NEAR INFRARED (NIR) SPECTROSCOPY FOR SELECTION OF MALTING BARLEY IN SOUTH AFRICAN BREEDING PROGRAMMES

by
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Declaration

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Abstract

Near infrared (NIR) spectroscopy in reflectance mode was evaluated for the prediction of barley quality properties (plumpness and moisture content) and malt quality properties (extract, total nitrogen (TN), total soluble nitrogen (TSN), Kolbach Index (KI), free amino nitrogen (FAN), diastatic power (DP), wort viscosity, apparent attenuation limit (AAL) and β-glucan content) from whole grain and ground South African malting barley, for quick evaluation of these properties in early stages of barley breeding programmes. Three different spectrometers (Büchi NIRFlex N-500, Bruker MPA and Büchi NIRLab N-200) and two data analysis software packages (The Unscrambler and OPUS) were used. Principal component analysis (PCA) allowed for distinction between irrigation and dry land samples, as well as samples cultivated at specific localities, based solely on sample spectra.

Whole grain calibration models appropriate for screening or rough screening were developed for the irrigation sample properties plumpness (r^2 = 0.52), extract (r^2 = 0.60), TN (r^2 = 0.78), TSN (r^2 = 0.50), KI (r^2 = 0.59), FAN (r^2 = 0.63) and wort β-glucan content (r^2 = 0.61), as well as for the dry land sample properties moisture content (r^2 = 0.53), extract (r^2 = 0.55), TN (r^2 = 0.79), TSN (r^2 = 0.71), FAN (r^2 = 0.77) and DP (r^2 = 0.72). Flour models that were acceptable for screening or at least rough screening were developed for the irrigation sample properties moisture content (r^2 = 0.69), plumpness (r^2 = 0.50), extract (r^2 = 0.55), TN (r^2 = 0.65), TSN (r^2 = 0.62), FAN (r^2 = 0.54), DP (r^2 = 0.58) and wort β-glucan content (r^2 = 0.54). Dry land flour sample models for moisture content (r^2 = 0.76), TN (r^2 = 0.84), TSN (r^2 = 0.59), FAN (r^2 = 0.60) and wort viscosity (r^2 = 0.65) were acceptable for screening or rough screening. AAL could not be predicted with accuracy appropriate for at least rough screening purposes. The use of variable selection showed improvement over the use of the entire spectral region in the case of whole grain models for dry land samples (moisture content, FAN and DP) as well as irrigation samples (extract, TN, KI and wort β-glucan content). Wort viscosity and β-glucan content were the only properties for which variable selection improved flour models. The addition of a second harvest season to calibration development did not show remarkable improvement on 2008 calibration models. Further improvement of these models requires expansion of sample ranges and the determination of malt properties from individual samples and not bulked samples.

PCA biplots were evaluated to illustrate the genotype-by-environment (GxE) interactions of malt properties for dry land and irrigation areas over the 2008 and 2009 harvest seasons. Consistency was observed over seasons and irrigation environments delivered more consistent quality for several properties over locations and seasons. Seasonal differences were also apparent and indicated that the GxE interaction should be studied over more than two seasons to determine if breeding lines can deliver consistent results for a specific locality or certain growing conditions. PCA biplots proved useful as an additional tool for visual evaluation regarding the quality of specific breeding lines over a series of localities and growing seasons.

Uittreksel

Naby infrarooi (NIR) spektroskopie is ge-evalueer vir die voorspelling van gars kwalitieit eienskappe (vetkorrel en vog inhoud) en mout kwaliteit eienskappe (ekstrak, totale stikstof (TS), totale oplosbare stikstof (TOS), Kolbach indeks (KI), vrye amino stikstof (VAS), diastatiese krag (DK), moutekstrak viskositeit, skynbare attenuasie limiet (SAL) en moutekstrak β-glukaan inhoud) van heelgraan en gemaalde Suid-Afrikaanse mout gars vir vinnige evaluasie van hierdie eienskappe in die vroe generasies van teel programme. Drie verskillende spektrofotometers (Büchi NIRFlex N-500, Bruker MPA and Büchi NIRLab N-200) en twee data analise sagteware pakette (The Unscrambler and OPUS) is gebruik. Hoof komponent analise (HKA) het toegelaat vir onderskeiding tussen besproeiings en droë land monsters, asook tussen monsters van spesifieke lokaliteite, gebaseer slegs op monster spektra.

Heelgraan kalibrasie modelle voldoende vir rofwegbepaling of sifting is ontwikkel vir die besproeiings monster eienskappe vetkorrel (r^2 = 0.52), ekstrak (r^2 = 0.60), TS (r^2 = 0.78), TOS (r^2 = 0.50), KI (r^2 = 0.59), VAS (r^2 = 0.63) en moutekstrak β-glukaan inhoud (r^2 = 0.61), asook vir die droë land monster eienskappe vog inhoud (r^2 = 0.53), ekstrak (r^2 = 0.55), TS (r^2 = 0.79), TOS (r^2 = 0.71), VAS (r^2 = 0.77) en DK (r^2 = 0.72). Vir meel monsters is modelle aanvaarbaar vir rofwegbepaling ontwikkel vir die besproeiings monster eienskappe vog inhoud (r^2 = 0.69), vetkorrel (r^2 = 0.50), ekstrak (r^2 = 0.55), TS (r^2 = 0.65), TOS (r^2 = 0.62), VAS (r^2 = 0.54), DK (r^2 = 0.58) en moutekstrak β-glukaan inhoud (r^2 = 0.54). Droë land meel monster modelle vir voginhoud (r^2 = 0.76), TS (r^2 = 0.84), TOS (r^2 = 0.59), VAS (r^2 = 0.60) en moutekstrak viskositeit (r^2 = 0.65) was aanvaarbaar vir rofweg bepaling. SAL kon nie voorspel word met geskikte akkuraatheid vir ten minste rofweg bepaling nie. Die gebruik van veranderlike seleksie het verbetering getoon op modelle waarvoor die volle spektrum gebruik is, in die geval van droë land heelgraan monsters (voginhoud, VAS en DK) asook besproeiings monters (ekstrak, TS, KI en moutekstrak β-glukaan inhoud). Moutekstrak viskositeit en β-glukaan inhoud was die enigste eienskappe waarvoor veranderlike seleksie meel modelle verbeter het. Die toevoeging van ‘n tweede oes seisoen vir kalibrasie ontwikkeling het nie merkwaardige verbeteringe op 2008 modelle alleen getoon nie. Verdere verbetering van die modelle benodig uitbreiding van monster reikwydte en die bepaling van mout eienskappe vanaf individuele monsters in plaas van monsters in grootmaat.

HKA bi-grafieke is ge-evalueer ter illustrasie van die genotipe-by-omgewing (GxO) interaksies van mout eienskappe vir droë land en besproeiings omgewings vir die 2008 en 2009 seisoen. Konsekwendheid is waargeneem oor seisoene en besproeiings omgewings het meer konsekwente kwaliteit vir verskeie eienskappe gelewer. Verskille is ook waargeneem oor seisoene en toon dat die GxO interaksie oor meer as twee seisoene bestudeer moet word om te bepaal of lyne konsistente resultate vir ‘n eienskap of in ‘n sekere omgewing (droë land of besproeiing) vir meer as een seisoen kan lewer. HKA bi-grafieke toon bruikbaar as ‘n addisionele visuele evaluerings metode ten opsigte van die kwaliteit van spesifieke teel lyne oor ‘n reeks lokaliteite en seisoene.

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Parkinson’s Law - the observed truth that work expands to fill the time available for its completion.

[*Parkinson* (1909 - 1993), Eng political scientist]

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List of Abbreviations

AACC	American Association of Cereal Chemists
AAL	Apparent attenuation limit
C	Carbon
ca.	*Circa* (approximately)
cP	Centipoise
CV	Cross-validation
DP	Diastatic power
EBC	European Brewery Convention
et al.	*et alii* (and others)
e.g.	exempli gratia (for example)
F1	First generation
F5	Fifth generation
FAN	Free amino nitrogen
Fig.	Figure
FT	Fourier-transform
g	Grams
H	Hydrogen
hrs	Hours
i.e.	*id est* (that is)
InGaAs	Indium Gallium Arsenide
IR	Infrared
J	Joules
KI	Kolbach Index
m	Meter
MC	Mean centering
mm	Millimeter
mg/L	Milligrams per litre
MIR	Mid-Infrared
MSC	Multiplicative scatter correction
n	Number of samples
N	Nitrogen
NIR	Near infrared
nm	Nanometres
O	Oxygen
PbS	Lead sulphite

PCA	Principal component analysis
PLS	Partial least squares
R^2	Coefficient of determination for calibration and cross-validation
r^2	Coefficient of determination for validation
r	Correlation coefficient
RMSECV	Root mean square error of cross validation
RMSEE	Root mean square error of estimation
RMSEP	Root mean square error of performance
RPD	Ratio of standard error of Prediction Validation to standard Deviation
S	Sulphur
SAB	South African Breweries
SABM	South African Breweries Maltings
SABBI	South African barley breeding Institute
SD	Standard deviation
SEC	Standard error of calibration
SEL	Standard error of laboratory
SEP	Standard error of prediction
SECV	Standard error of cross validation
S/N	Signal-to-noise ratio
SNV	Standard Normal Variate
TN	Total Nitrogen
TSN	Total Soluble Nitrogen
USDA	United States Department of Agriculture
W.K.	Windisch Kolbach units

Chapter 1

Introduction

Chapter 1

Introduction

Barley (*Hordeum vulgare*) is a major world crop and has the ability to adapt and survive in a wide range of environmental conditions. Ongoing breeding processes, from as early as the 1800's in Europe and the 1900's in North America, have improved barley quality and productivity immensely (Nilan & Ullrich, 1993). After wheat, barley is the most important small grain in South Africa (Kotze, 2009) and is used mostly for the production of malt (Poehlman, 1985; Ullrich, 2002). Barley malt is mainly used as a source of fermentable sugars for alcoholic fermentation for the production of beer and whisky (Bamforth & Barclay, 1993; Kreisz, 2009).

Malt is barley that has been allowed to germinate for a limited period of time and then dried (Hough, 1991; Kreisz, 2009). The process of malting is related to a series of biochemical changes that occur during germination. Germination occurs when moisture and oxygen (supplied by the atmosphere) are present and temperature conditions are appropriate (Hunter, 1962). A maltster gathers suitable stocks of barley and then steeps the grain in water, allowing germination to occur. During germination, the food store in the endosperm of the kernels, which is available to support the development of the germ of the grain, is partly degraded by enzymes (Briggs *et al*., 1981; Bamforth & Barclay, 1993). The physical degradation of the endosperm and the resulting biochemical degradation is known as modification; a term used to describe the extent of enzymatic degradation (Hough, 1991; Kreisz, 2009). After modification (germination) has proceeded to a desired extent, the grain is dried at a low temperature followed by a higher temperature, causing a suspension of the enzyme activity (Schuster, 1962; Kreisz, 2009). Malt is the product remaining after the shriveled and brittle rootlets fall off.

Barley malt is a basic raw material in the brewing process and it is important that the quality of the malt is of a certain standard. The malting and brewing industries have related desired qualities in malt with certain properties of the raw barley grain for centuries which has lead to the belief that good beer can only be made from good malt, and good malt can only be made from good barley (Meredith *et al*., 1962; Burger & LaBerge, 1985; Savin & Molina-Cano, 2002).

The barley breeder aims to produce cultivars with desirable agronomical properties such as high yield and disease resistance as well as high enzyme content (Schuster, 1962). Maltsters require barley that germinates homogeneously, modifies quickly and will deliver malt of acceptable and consistent quality. Therefore, maltsters set certain quality standards for malting barley (Burger & LaBerge, 1985; Kotze, 2009). There exists no single gene for optimal barley quality, and breeding thus utilizes a mixture of genes, combining advantageous traits together in one plant (F. Potgieter, South African Barley Breeding Institute (SABBI), Caledon, South Africa, Personal Communication, 2009). Breeding of new malting barley cultivars requires the evaluation of many grain characteristics contributing to malt quality and, in most cases, the complexity of these analyses limit the number of samples that can be tested. Evaluation is thus mainly carried out prior

to release of a new cultivar, as thorough evaluation is only possible if small numbers of lines are to be tested and an adequate amount of sample is available. Various properties are important in the determination of barley, as well as malt quality and require larger numbers of lines to be evaluated at earlier stages in the breeding programme (Meredith *et al.*, 1962; Henry, 1985a; Savin & Molina-Cano, 2002). Methods previously used for the prediction of barley malting quality are extremely diverse (Henry, 1985a) and are all aimed at predicting malting quality rapidly with the use of small sample sizes (Bamforth & Barclay, 1993).

The barley quality properties that are especially important to breeders and farmers are its moisture and nitrogen contents. Barley, delivered and stored with a high moisture content, can lead to fungal development and a decrease in germination capacity (Kotze, 2009). Nitrogen content is universally regarded as a major quality factor (Pollock, 1962) since there is a clear association between poor malting quality and high nitrogen content of barley (Meredith *et al.*, 1962; Bamforth & Barclay, 1993; Kotze, 2009). Barley cultivars which modify well are generally plump and well filled, while poor quality is associated with thinness (Meredith *et al.*, 1962; Kotze, 2009).

The major component of the endosperm cell wall is β-glucan (Fincher & Stone, 1993). High β-glucan levels in a malt sample is undesirable in the malting and brewing process as it indicates incomplete cell wall degradation (Duffus & Cochrane, 1993), which will result in lower malt extract values (Fincher & Stone, 1993); brewers demand high quality malts capable of delivering high extract yields (Henry, 1985a; Kotze, 2009). The measure of the viscosity of wort provides useful information about β-glucan content and the degree of modification of the malt (Pollock, 1962; Anger *et al.*, 2009).

The potential of malt to provide fermentable sugars and nitrogenous compounds to be used by yeast is among the most important of its properties. Soluble nitrogenous compounds are a food source for yeast used in brewing (Pollock, 1962). The total nitrogen (TN) content of malt is comparable to the barley from which it was obtained (Bamforth & Barclay, 1993), while nitrogenous materials such as amino acids and peptides are known as free amino nitrogen (FAN). High FAN levels are unwanted, as it diminishes the microbiological stability of the final beer product (Bamforth & Barclay, 1993). The level of FAN influences the extent of fermentation by yeast (Kotze, 2009) and for beer, it is necessary to achieve a constant degree of attenuation; the limit to which fermentation proceeds, also known as the apparent attenuation limit (AAL) (Bamforth & Barclay, 1993). An important property to maltsters is the amount of nitrogenous material that can be extracted from ground malt with warm water (wort), referred to as total soluble nitrogen (TSN) (Hough, 1991; Bamforth & Barclay, 1993). The soluble nitrogen ratio, also referred to as Kolbach Index (KI), is the soluble nitrogen as a percentage of the TN (Hough, 1991; Bamforth & Barclay, 1993) and brewers demand the KI to be sufficiently high to indicate that adequate protein modification has been achieved (Bamforth & Barclay, 1993; Kotze, 2009).

The ability to convert starch to fermentable sugars is an important quality factor, and diastatic power (DP) reflects the action of four starch degrading enzymes including α-amylase, limit-

dextrinase, α-glucosidase and β-amylase (Duffus & Cochrane, 1993; Shewry & Darlington, 2002). The action of these enzymes on starch leads to rapid decreases in viscosity and to the formation of simple sugars (Pollock, 1962; Duffus & Cochrane, 1993).

The ability to predict barley quality for malting purposes in early generations would be of great benefit to breeders and maltsters, allowing for selection of suitable lines to deliver malt of the highest quality. In current South African breeding programmes, limited quality evaluation is carried out by means of near infrared (NIR) spectroscopy in transmittance mode for evaluation of barley moisture and nitrogen content. At later stages in the breeding programmes, micro-malting can be carried out but this technique requires large sample sizes, is destructive and requires experienced personnel (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). Thus, the need for effective quality evaluation is still essential and most analytical methods are time consuming and laborious. NIR spectroscopy is an ideal technique for this purpose as it is fast, reliable and requires no highly skilled personnel (Osborne, 1981). NIR spectroscopy offers the potential to conduct rapid tests, non-destructively, on a small sample of whole grain for quality evaluation in early generations where limited seed is available (Woodcock *et al.*, 2008).

NIR spectroscopy has been used for quality testing in cereal breeding programmes since the late 1970's (Osborne, 2006) and is well-established for the determination of protein and moisture in wheat and durum breeding programmes. The application of NIR spectroscopy is based on the empirical relationship between reference data obtained by analytical methods, and the spectral data obtained with a spectrophotometer, to acquire quantitative and qualitative information from the interaction between NIR electromagnetic waves and the chemical components of the sample (Pasquini, 2003). The NIR range spans from 750-2500 nm (Butler, 1983) and NIR instruments can be used in transmittance and reflectance modes; instruments operating in reflectance mode use the wavelength range 1100-2500 nm.

Studies regarding the prediction of barley and malt quality characteristics with NIR reflectance spectroscopy, both on whole and ground barley grain and malt, as well as on wort, have delivered suitable prediction models. These include whole grain barley analyses for plumpness (Edney *et al.*, 1994), moisture content (Downey, 1985; Halsey, 1987; Li *et al.*, 1995; Sohn *et al.*, 2008), β-glucan content (Black & Panozzo, 2001; Sohn *et al.*, 2008), nitrogen content (Halsey, 1987; Edney *et al.*, 1994; Li *et al.*, 1995; Sohn *et al.*, 2008), DP (Li *et al.*, 1995; Black & Panozzo, 2001), FAN, TSN (Black & Panozzo, 2001) as well as extract (Halsey, 1987; Li *et al.*, 1995; Black & Panozzo, 2001) and wort viscosity (Li *et al.*, 1995). Studies on ground barley have included the prediction of nitrogen (Gill *et al.*, 1979; Henry, 1985b) and moisture contents (Downey, 1985; Henry, 1985b), β-glucan content (Allison *et al.*, 1978; Henry, 1985b; Szczodrak *et al.*, 1992) as well as malt extract (Morgan & Gothard, 1979; McGuire, 1982; Henry, 1985b). Studies conducted on whole grain malt for the prediction of malt quality included the parameters extract, DP, β-glucan content, FAN and TSN (Black & Panozzo, 2001), while studies conducted on ground malt have included moisture (Henry, 1985c; Marte *et al.*, 2009) and nitrogen contents (Marte *et al.*, 2009) as well as extract

(Henry, 1985c). Studies have also been done on wort including the prediction of extract, FAN and TSN (Ratcliffe & Panozzo, 1999).

Seasonal differences in the quality of malting barley are a matter of common knowledge to farmers and maltsters (Savin & Molina-Cano, 2002), while soil and climate are dominating influences in the character of malting barley (Hunter, 1962). Selection of lines with superior quality from various localities is thus not simple, due to the presence of genotype-by-environment (GxE) interactions, i.e. differential genotypic expression across environments. GxE has important implications in breeding programmes, including specific adaptation and choice of location for selection as well as resource allocation in advanced line testing across locations and years (Voltas *et al*., 2002). Several GxE studies regarding malting barley have been conducted; studies in Spain included the malt properties extract, TN, TSN, KI, wort viscosity and AAL. Results showed significant genotype, locality and year effects (Molina-Cano *et al*., 1997). Eagles *et al.* (1995) found highly significant interactions for genotypic and environmental correlations of nitrogen, malt extract and DP in South East Australia. Den Hartog and Lambert (1953) found protein, DP and extract to be closely related while a study in Poland confirmed a high GxE interaction for KI and extract (Kaczmarek *et al*., 1999). The investigation of GxE effects on DP proved that both have an influence on this property (Arends *et al*., 1995). The assessment of the effect of cultivar and environment on β-glucan content of barley revealed that cultivar was the most significant influential factor (Oscarsson *et al*., 1998).

Although numerous reports regarding NIR studies on malting barley and studies of GxE interactions exist, no information was found for barley in a South African breeding programme and therefore the aim of this study was to:

- develop NIR calibration models for the prediction of quality properties for malting barley (moisture content, plumpness) as well as for malt (extract, TN, TSN, KI, FAN, DP, wort viscosity, AAL and wort β-glucan content), both from barley; and to
- study the GxE interaction in relation to the quality characteristics of malting barley with regard to different localities and lines over two growing seasons (2008 and 2009).

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Chapter 2

Literature review

Contents

1. Introduction

Barley malt is the main raw material and the main starch source for brewing worldwide. Barley (*Hordeum vulgare*) is a highly specialized cereal with a long breeding, malting and brewing tradition; quality specifications for brewing barley are the most challenging specifications in comparison to other cereals in the food industry (Kreisz, 2009). The cultivation area for malting barley in South Africa is restricted to two very specific regions; where it is grown under irrigation in the Northern Cape and under dry land conditions in the Southern Cape (**Fig. 2.1**) (Kotze, 2009a). Every year, approximately 90 000 and 150 000 tons of barley is produced in these regions, respectively (F. Potgieter, South African Barley Breeding Institute (SABBI), Caledon, South Africa, Personal Communication, 2009).

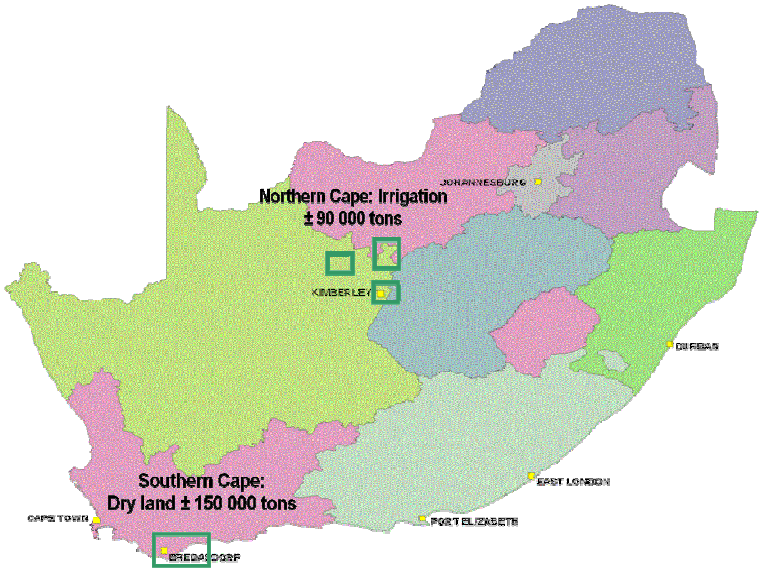

Figure 2.1 Graphical representation of dry land and irrigation regions (indicated by green blocks) in South Africa (Supplied by SABBI).

The fact the production of malting barley is restricted to these specific areas is advantageous with respect to transport, storage and research (Kotze, 2009a). However, the problem arises in the selection of suitable cultivars for each of these regions that meet required quality specifications. Breeding of new cultivars therefore requires the evaluation of many quality characteristics and the testing and selection of thousands of breeding lines, starting with early generation material; many tests require larger samples of barley than are available in earlier generations of the breeding programme. Near infrared (NIR) spectroscopy is an ideal technique for this purpose as it is fast, reliable, non-destructive and does not require large sample sizes (Osborne, 1981). The purpose of a NIR instrument is to estimate the concentration of chemical properties (such as protein content), quickly and precisely from spectrophotometric measurements. This allows it to replace slower, expensive or more imprecise methods for assessing the desired chemical constituents.

The small cereal grains were among the first commodities used in the development of NIR instruments and methods (Delwiche, 2004) and cereal chemists in breeding programmes were among the first to identify the potential of NIR spectroscopy to replace conventional laboratory quality tests. NIR spectroscopy has been used for quality testing in cereal breeding programmes since the late 1970's (Osborne, 2006). The NIR technique is very simple and, once suitable calibrations have been developed, sample analysis takes no longer than a minute. This technique would allow plant breeders to predict barley and malt quality from unmalted barley to select cultivars that perform well, both in the field and in the malt house and thereby increase the amount of high quality breeding lines in later generations.

Variation in barley quality from year to year is a major problem in the brewing industry and can mostly be attributed to genotype-by-environment (GxE) interactions (Savin & Molina-Cano, 2002). GxE interactions have important implications in breeding programmes (Voltas *et al*., 2002) as differential genotypic expression across environments cause difficulties in the selection of cultivars with superior quality. For trials in which genotypes and locations are repeated for years, genotype by location and genotype by year interactions can be assessed via the use of statistical techniques such as analysis of variance (ANOVA) and Principal Component Analysis (PCA) biplots. The data are generated through a series of trials carried out over sufficient locations and years to represent the target area for the breeding programme (Voltas *et al*., 2002).

This literature study will review malting barley, barley breeding and the malting process. The biochemistry of malting barley and important barley and malt quality factors will be discussed, followed by a review of NIR spectroscopy. Studies regarding the prediction of malting barley quality with NIR spectroscopy will also be reviewed and lastly, a short discussion on the influence of GxE interactions on malting barley is given.

2. Malting barley

The barley kernel is a complex integration of carbohydrates, proteins, lipids, minerals and other compounds (Savin & Molina-Cano, 2002). At harvest, the moisture content of the barley grain is approximately 14% with 3% lipids and 2% minerals (Duffus & Cochrane, 1993). Carbohydrates are the major components (70-80%) of the barley grain (Duffus & Cochrane, 1993; Ullrich, 2002; Kreisz, 2009) with starch as the primary component (50-70% of dry weight (Kreisz, 2009)). Protein may account for 8-15% of dry weight and β-glucans for 3-6% (Duffus & Cochrane, 1993; Shewry, 1993; Savin & Molina-Cano, 2002). In unmalted barley, β-amylase may account for 1-2% of total protein in the starchy endosperm. Variations in proportions are due to differences in genotype and environmental conditions as well as measuring techniques (Duffus & Cochrane, 1993; Savin & Molina-Cano, 2002).

The ideal malting barley can be referred to as ripe, plump kernels with a reticulated skin and relatively low protein content and contains a white mealy endosperm from which starch granules can be readily removed (Poehlman, 1985). For maltsters and brewers, starch and protein contents

are the most important constituents to consider. Structural differences may occur in the endosperm of the barley grain and can be visually classified as mealy or steely (Ferrari *et al*., 2010); unripe grain usually shows a smooth, unwrinkled skin, and when broken will exhibit a dark, steely endosperm surface (Briggs *et al*., 1981a). The latter grain modifies slowly during malting and produces unsatisfactory malt due to a higher nitrogen content (Briggs *et al*., 1981a; Ferrari *et al*., 2010).

3. Breeding of malting barley and breeding objectives

Feed is the primary use of barley grain around the world but recently, the majority of barley quality improvement research and breeding has been directed toward malting barley despite its minority use (Ullrich, 2002). Malting barley commands a premium price over feed barley and in South Africa malting barley is sold for R3500/ton, while feed barley sells for R1500/ton (I. Meijering, South African Breweries Maltings (SABM), Caledon, South Africa, Personal Communication, 2009). Feed types are designated because they do not posses acceptable malting quality characteristics and not because they posses particular feed or nutritional characteristics (Ullrich, 2002), although studies have proven that malting barley has the ability to exhibit excellent feed characteristics (Fox *et al*., 2009). Economically, cultivar choice is an important decision for farmers, and factors that determine cultivar choice are fundamental (Kotze, 2009a). No cultivar with all desirable attributes has yet been obtained through breeding, and breeders still need to introduce properties from different cultivars for specific agricultural and commercial requirements.

New cultivars are bred by crossing existing cultivars to combine advantageous traits in one plant (Bell & Lupton, 1962) and this is achieved by manual cross-pollination between cultivars. Barley lines with desired characteristics are then sought out in the descendants of these crosses, and new cultivars are accepted for commercial production when trials show them to be superior to current cultivars in terms of yield, disease resistance, agronomic characteristics and malting quality (Briggs *et al*., 1981a). With this technique, the breeder is able to control the resulting population to some extent and can choose the parents which he crosses, to give maximum chance of the population showing the necessary qualities. In its simplest form, it involves the crossing of two parental cultivars, and it is necessary to provide a sufficient number of hybrid grains for raising F1 plants which will in turn provide an adequate population in the F2 generation (Bell & Lupton, 1962).

SABBI follows an 18 year breeding programme; 150 initial crosses of selected parents are made each year and planted at the SABBI breeding nursery in Caledon (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). The F1 generation consists of 4000 new lines every year. Three years of single plant selection follows where desired plants are visually selected for agronomical and barley quality characteristics. From F5 onwards selected plants are planted in 6 m x 1 m rows. Approximately, 100 g of seed can be obtained at this stage of the breeding programme. From the F5 generation, 1000 lines are selected, which is drastically reduced during the next four years in Elite trials. At this stage selected plants are grown at different locations in the

dry land and irrigation areas and three replicates of each line are planted using a nearest neighbour design. Experimental release trials are carried out from years 13 to 16, followed by the final stages of the breeding programme: seed multiplication and commercial production (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). Breeding therefore requires the selection of suitable cultivars and involves the evaluation of thousands of lines starting with early generation material (Osborne, 2006).

The improvement of cultivars, by breeding, must consider agricultural, processing and consumer interests (Bell & Lupton, 1962). The breeder must identify his aims, taking the interests of the farmer, who grows the barley for processing, into consideration. The interests of the farmer coincide with those of the processor. Breeding objectives are determined by agricultural, ecological and economic circumstances (Bell & Lupton, 1962) with the main aim to produce cultivars with desirable agricultural properties such as high yield, disease resistance (Schuster, 1962) and drought tolerance as well as improved nutritional or feed quality (Nilan & Ullrich, 1993). The development of improved cultivars for better malting quality is also of great importance (Nilan & Ullrich, 1993) since maltsters demand that barley has a high enzyme potential (Schuster, 1962) and low levels of fibrous materials and total nitrogen (Briggs *et al.*, 1981c).

There is no simple clear group of variables that is unanimously regarded as defining the quality of grain or malt (Savin & Molina-Cano, 2002). Rather, quality requirements in malting barley represent a consensus of the specifications required by commercial brewers to produce their products in an efficient manner consistent with desired product properties. Moreover, brewers have different specifications and there are certain ranges for each quality factor within which each brewer can operate by adjusting conditions or by tolerating some variation in the product (Burger & LaBerge, 1985).

4. Malting

Malt is germinated barley; the most important use of which is as a source of fermentable sugars for alcoholic fermentation (Bamforth & Barclay, 1993). Malting is concerned with the modification of the grain and is described as the “liberation of starch granules from the matrix of the cells of the endosperm in which they are embedded” (Hunter, 1952). During the early stages of the malting process, the cell walls of the endosperm are dissolved through the action of hydrolytic enzymes, permitting the diastatic enzymes to come into contact with starch granules, which are then liberated from the protein matrix (Hunter, 1962; Bamforth & Barclay, 1993). About 50% of total barley protein is mobilized during malting, the extent of this proteolysis, in conjunction with that of cell wall degradation, depends on the precise steeping and germination conditions employed (Bamforth & Barclay, 1993).

Barley is cleaned and graded before malting and extraneous matter is removed. The high quality demanded is specified by several quality parameters like germination capacity, protein content, kernel size, moisture content, kernel abnormalities and infestation (Kreisz, 2009). If the

moisture content of the barley is more than 15%, it must be carefully dried before storage to inhibit the development of fungi and bacteria (Schuster, 1962).

The most important factors to consider when making malt are the quality of the finished malt, the yield of malt from a given quantity of barley and the efficiency of the process with respect to labour and energy (Schuster, 1962). Commercial malting operations involve five basic steps: barley intake, drying and storage; steeping; germination; and kilning (Bamforth & Barclay, 1993).

4.1 Steeping

The steeping operation is the most important stage in malting. For production of homogenous malt, an even moisture content must be achieved across the grain bed (I. Meijering, SABM, Caledon, South Africa, Personal Communication, 2009) to activate metabolism in the embryonic and aleurone tissues, leading to the development of hydrolytic enzymes (Bamforth & Barclay, 1993). Water saturation of the starchy endosperm is also critical before the food reserves of that tissue can be mobilised through enzyme action (Bamforth & Barclay, 1993), as high moisture content results in faster modification (Briggs, 1978). The water uptake mainly depends on the steep water temperature and the duration of the wet periods. Higher steeping water temperature and long wet periods increase the water uptake, but the risk of drowning water-sensitive barley is higher and microbial growth is accelerated (Kreisz, 2009). Conditions during steeping must account for the nature of the barley, including kernel size and nitrogen content. Moisture content required for germination varies with barley supply and steeping involves submerging grain in water for 43 hrs until the moisture content has reached a desired level of 46% (Bamforth & Barclay, 1993).

A clean barley batch is immersed in water at approximately 14 to 17˚C in a steep tank, forming a 3 m thick bed (Hough, 1991; Kreisz, 2009). Water enters the barley at the base (embryo end) of the kernel and spreads through the endosperm until evenly distributed. The rate of water absorption is rapid during the first few hours of steeping and slows down gradually as the saturation level is approached (Schuster, 1962). During steeping, the embryo and husk take up water rapidly while the starchy endosperm hydrates more slowly (Hough, 1991; Bamforth & Barclay, 1993; Kreisz, 2009). The mealy endosperm of malting barley contains many cracks with starch granules loosely packed in the protein matrix which allows water to diffuse more readily (Bamforth & Barclay, 1993).

When barley absorbs water during steeping, the embryo uses oxygen dissolved in the steeping water for respiratory purposes (Schuster, 1962). Steeping is interrupted by draining after 12 to 24 hrs, a step known as air rest (Hough, 1991). Air rest removes carbon dioxide and ethanol, which may inhibit germination. These substances are produced as a result of respiratory metabolism in the embryo and aleurone tissues, as well as through the action of microorganisms on surface tissues (Bamforth & Barclay, 1993). In the air rest period, the grain is coated with a film of moisture; air is forced downward through the bed to help disturb this film, introducing oxygen and eliminating carbon dioxide (Bamforth & Barclay, 1993).

Air is forced through the steep water using perforated pipes or is pulled through by suction (Hough, 1991). This adds oxygen which is needed by the kernels for respiration. A lack of oxygen may provoke CO_2 accumulation followed by fermentation and consequently poisoning of the germ (Kreisz, 2009).

After the air rest period, the barley is re-immersed in water. This alternation of steep water and air rest continues until the barley has reached a moisture content of 46% (Hough, 1991). A typical steeping process may involve an initial steep to 32% moisture; the start of germination is promoted by an air rest of 10 to 20 hrs (Bamforth & Barclay, 1993). At this stage of the process, the kernel changes visually by developing a small white root at the base of the kernel called a chit (Hough, 1991; Kreisz, 2009). Chitting is encouraged with a second air rest of 10 to 15 hrs before the final steep (Bamforth & Barclay, 1993) to raise the moisture to 46% (Bamforth & Barclay, 1993; Kreisz, 2009). Homogenous chitting of all kernels can be checked visually and is essential for a homogenous malt quality (Kreisz, 2009).

4.2 Germination

Malting involves germination of the grain until the endosperm has been degraded by enzymes to be mobilised for development of the germ (Briggs *et al.*, 1981c). Germination is targeted at generating the maximum available extractable material by promoting endosperm modification through the development, distribution and action of enzymes (Bamforth & Barclay, 1993). The maltster is therefore concerned with both the degradation of the endosperm and the mobilisation of the enzymes of the grain. Growth of the germ or embryo is incidental to the production of malt but excessive growth leads to depletion of the endosperm material through metabolism for the embryo. The maltster makes use of the natural germination process but only allows it to proceed until enzyme activity is optimal and terminates the degradation of the endosperm and growth of the embryo by drying the grain (Schuster, 1962). The objectives of germination are optimal production levels of hydrolytic enzymes, controlled breakdown of cell walls and matrix proteins, hydrolyzation of certain barley reserves (protein to form free amino nitrogen (FAN)), minimizing loss of potential extract from growth and respiration while achieving optimal modification, and produce balanced, well-modified green malt for kilning (Kreisz, 2009).

After the steeping process, the water is drained off and the barley is spread out as a malt bed (approx 1.5 m deep) where it will germinate. Cool humidified air is pushed through the bed at 14 to 17˚C (I. Meijering, SABM, Caledon, South Africa, Personal Communication, 2009). Soon after germination begins, a synthesis/activation of hydrolytic enzymes occur, allowing for the development of an extensive root system (Kreisz, 2009) at the base of the grain, beneath the husk. Gibberellic acid induces the production of many different hydrolytic enzymes in the aleurone layer which covers the whole endosperm (Kreisz, 2009).

The length of acrospire (first leaf) growth in relation to the length of the grain may be used to evaluate the homogeneity of the batch, where different acrospire lengths and very long acrospires

indicate heterogeneity in growth and therefore a non-homogenous malt quality. The enzymes are synthesised by the aleurone cells (Bamforth & Barclay, 1993) and migrate through the starchy endosperm, progressing from the embryo (proximal) end of the kernel (Kreisz, 2009). Respiration occurs and the starchy material of the endosperm is used for this purpose. Amylase will degrade starch and degradation products are used by the embryo as a source of energy for growth. Thus the β-amylase originally present in barley allows for only a slight degradation of starch but with the activation of α-amylase during the early days of germination a more noticeable breakdown of this polysaccharide occurs (Schuster, 1962). This mobilisation phase is known as 'modification' (the term used to describe the extent of endosperm modification) (Hough, 1991).

The cell walls and protein matrix of the starchy endosperm are degraded by hemicellulases and proteases respectively, exposing the starch granules and resulting in "mellowness" of the malt (Schuster, 1962; Bamforth & Barclay, 1993).

During the germination process, the grains are turned at intervals to prevent the rootlets from matting together. The rate of modification depends on the rate at which moisture distributes through the starchy endosperm; the rate of enzyme synthesis; the extent of release of these enzymes into the starchy endosperm; and structural features of the endosperm that might be resistant to degradation (Bamforth & Barclay, 1993). The products of endosperm breakdown (sugars, amino acids) together with materials from the aleuronic layer (phosphate, metal ions) are needed for growing the germ. It is now a challenge for the maltster to control the hydrolysis of proteins (proteolysis), cell walls (cytolysis) and starch (amylolysis) (Kreisz, 2009).

The germination process is controlled by maintaining a constant moisture level within the grain, supplying oxygen and removing carbon dioxide while also eliminating heat formed by respiration. The grain is turned mechanically every 8 to 12 hrs, as well as immediately before kilning (Bamforth & Barclay, 1993). Germination is performed at 14 to 17°C for four days (I. Meijering, SABM, Caledon, South Africa, Personal Communication, 2009). During germination, moisture is transferred from the malt to the surrounding air and the embryo withdraws moisture from the starchy endosperm to sustain its growth, causing it to dry out (Bamforth & Barclay, 1993). This interferes with modification (Bamforth & Barclay, 1993) and the water content of the grain should remain constant during germination (Schuster, 1962).

4.3 Kilning

After the endosperm has been sufficiently degraded (allowing for even modification), the malt is kilned to terminate embryo growth and endosperm degradation (Schuster, 1962; Kreisz, 2009) and to reduce moisture levels to less than 5% (Bamforth & Barclay, 1993; Kreisz, 2009). The most important aim of kilning is to fix those sought-after properties obtained in the malt during germination, and in addition, allow for development of flavour and aroma characteristics in the malt (Schuster, 1962; Kreisz, 2009). This process must be carefully regulated to conserve enzyme complexes developed during malting that will hydrolyze the malt starch into fermentable sugars

during brewing (Bamforth & Barclay, 1993; Kreisz, 2009) and enables the maltster to store the dried malt for long periods of time, e.g. six months, in a stable state (Schuster, 1962; Kreisz, 2009).

Kiln drying is divided into four main phases: free drying to 23% moisture; an intermediate stage of drying to 12% moisture; the bound water stage from 12 to 6%; and curing, in which the moisture is taken to 3 to 5% (Bamforth & Barclay, 1993).

The basic principles of kilning are that drying should commence at a relatively low temperature to ensure survival of the most heat-sensitive enzymes (limit-dextrinase, β-glucanase) followed by an increase of temperature to ensure flavour and colour changes and complete drying in less than 24 hrs (Bamforth & Barclay, 1993). There is firstly a drying period where water is removed from the malt at 50 to 60˚C (Schuster, 1962; Hough, 1991). Then follows the curing process carried out at higher temperatures of approximately 80℃, during which a further 3 to 4% of water is removed (Schuster, 1962). The grains are then screened allowing the shriveled and brittle rootlets to fall off. The remaining product is malt.

5. Biochemistry of malting grain

Malt is, in appearance, much like barley but changes occur in barley during the various malting processes. An illustration of the longitudinal section of the barley grain is shown in **Fig. 2.2**. During steeping, the grain swells and increases in volume. The first indication of germination is the appearance of the white chit (the root sheath) which protrudes from the base of the kernel, beyond the husk. After this, seminal roots (rootlets) break through the root sheath and form a tuft at the end of the grain. Meanwhile, the coleoptile, with its enclosed first leaf (acrospire), penetrates the testa on the dorsal side of the grain and grows towards the apex between the testa and the pericarp. This leads to the breakdown of the starchy endosperm and degradation of the cell walls in the intact grain. The growth of the acrospire is often used as a guide to indicate the progress of the malting process (Briggs *et al.*, 1981b).

Following cell wall breakdown, the proteins of the endosperm undergo degradation and the starch granules are partially degraded. All these changes are termed ‘modification’ and are catalysed by hydrolytic enzymes (Briggs *et al.*, 1981b). During this stage, changes occur in the cells of the aleurone layer and scutellar epithelium, which are associated with the mobilization of the cell’s reserves, the synthesis and release of hydrolytic enzymes and the uptake of soluble substances (sugars, amino acids and minerals that serve as nutrients for the growing embryo) from the starchy endosperm and aleurone layer (Briggs *et al.*, 1981b).

The endosperm of ungerminated barley is tough, but after germination, the moist endosperm is friable, because the process of modification reduces the strength of the endosperm and changes its character. During malting, it is the synthesis of hydrolytic enzymes and the breakdown of the structural components of the starchy endosperm which constitute modification. The material remaining at the end of malting delivers the brewers’ extract (Briggs *et al.*, 1981b).

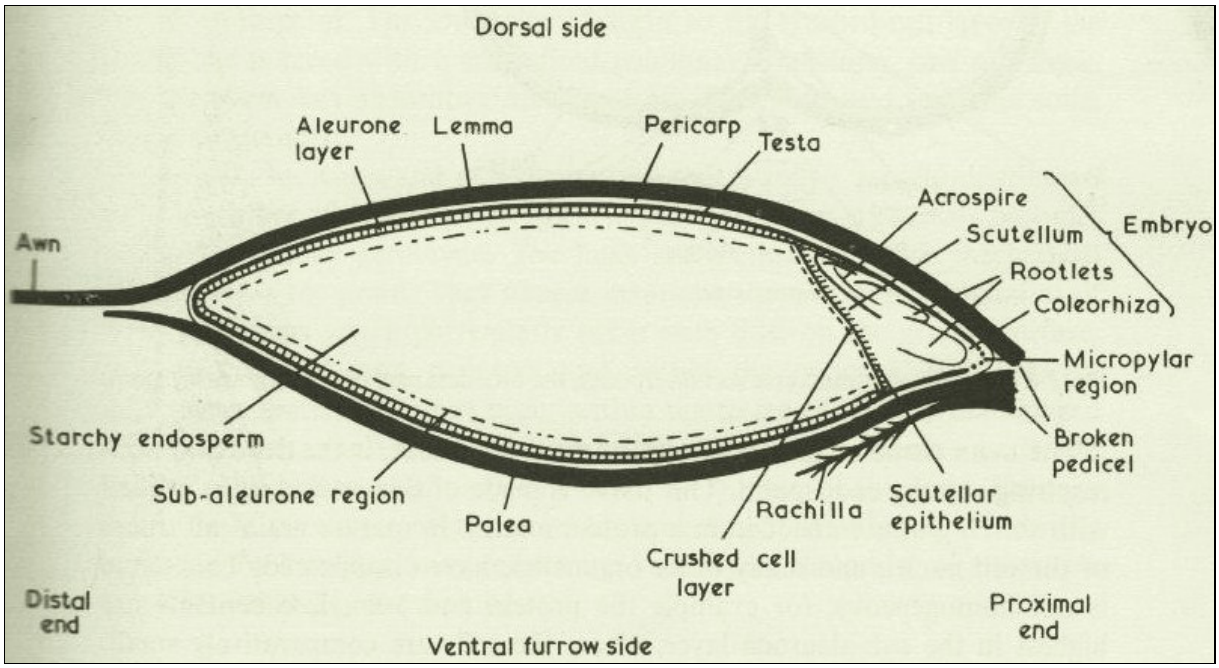

Figure 2.2 Longitudinal section of barley grain (Hunter, 1962).

6. Quality factors

Breeding of new malting barley cultivars requires the evaluation of many grain characteristics that contribute to malt quality (Henry, 1985b; Savin & Molina-Cano, 2002). Malt quality is directly influenced by the raw barley grain it is obtained from, and therefore, quality evaluation of the barley grain is imperative to breeders. Since malt is one of the main raw materials in the brewing process its quality must be rigidly assessed in order to satisfy the requirements of the brewing process (Meredith *et al.*, 1962; Savin & Molina-Cano, 2002). Malt properties are determined on malted barley that has been ground to form flour with many components but mostly rich in starch, and enzymes (diastase) capable of acting rapidly when hot water is added. The resultant liquid is known as wort, the sweet liquor from which beer is made (Hunter, 1962; Briggs *et al.*, 1981c). In South Africa, malt is mashed to produce wort in accordance with the method in Analytica European Brewery Convention (EBC) Methods Manual, Section 4.9.1 (European Brewery Convention, 1998).

6.1 Barley quality

6.1.1 Kernel nitrogen content

The nitrogen content of barley is regarded as a major quality factor since the amount and composition of barley proteins have an important influence on grain quality and its suitability for malting (Pollock, 1962). Barley protein accounts for 8 to 15% of the dry weight of a mature barley grain (Shewry, 1993).

There is a clear association between poor malting quality and high nitrogen content of barley (Meredith *et al.*, 1962; Foster *et al.*, 1967; Arends *et al.*, 1995; Eagles *et al.*, 1995; Molina-Cano *et al.*, 1997). Barley with extensively high or low nitrogen content cannot produce malt of the required

quality for brewing purposes (Kotze, 2009b) and steely grains are often higher in nitrogen and malt less readily than mealy barleys (Briggs *et al*., 1981a). High nitrogen content leads to decreased level of carbohydrates (starch). High nitrogen content also leads to an increase in the time necessary for modification in the malt house, incomplete modification and an increase in malting losses due to excessive acrospire and rootlet growth (Burger & LaBerge, 1985).

In South Africa, the price for malting barley increases as the nitrogen increases from 1.50 to 1.74% and an increased premium is paid for barley with nitrogen content between 1.75% and 1.85% (on dry basis). The price decreases as the nitrogen increases from 1.86 to 2.00% (Kotze, 2009b). Barley nitrogen content is determined according to EBC method 4.3.1 or 4.3.2 (European Brewery Convention, 1998).

6.1.2 Moisture content

Storage of barley with an excessively high moisture content (more than 14%) can lead to fungal development and decreased germination capacity (Burger & LaBerge, 1985; Kotze, 2009b). Wet barley respires more rapidly than dry barley, using oxygen and producing heat, water and carbon dioxide. The rate of respiration increases with increasing moisture content and goes up rapidly with moisture contents above 13%. Moist grain is susceptible to attack by many insect pests and spoilage fungi, especially at elevated temperatures (Briggs *et al*., 1981a). For this reason, in South Africa, malting barley with a moisture content higher than 13% is not accepted by maltsters and more is paid for grain with moisture content decreasing from 13% to 9.5% (Kotze, 2009b).

Drying ground samples to constant weight provides direct evidence of grain moisture content, but resulting values are highly dependent on the method (Pollock, 1962). Barley moisture is determined according to the method in the Analytica EBC Methods Manual, Section 4.2 (European Brewery Convention, 1998).

6.1.3 Plumpness

Barley kernels which modify well are plump and well filled, while poor quality is associated with kernel thinness (Meredith *et al*., 1962). Thin kernels have a higher proportion of husk (Kotze, 2009b) which is composed of cellulose and does not contribute to malt extract. Therefore, the larger the ratio of the interior (endosperm) to the husk, the greater the quantity of extract that will be obtained (Hunter, 1952). Plumpness is important for homogeneity during the malting process and since thin kernels take up water faster than plump kernels, a more uniform plumpness will result in better malt quality. Maltsters pay more for barley with a kernel plumpness increasing from 70 to 100%, measured above a 2.5 mm sieve (Kotze, 2009b; I. Meijering, SABM, Caledon, South Africa, Personal Communication, 2009).

6.2 Malt quality

6.2.1 Extract

The extract percentage indicates the maximum soluble yield obtained from a specific malt and is the most frequent parameter analyzed in wort (Anger *et al.*, 2009). The higher the extract the more soluble the material and less husk and protein is present (Kotze, 2009b). The ability of barley grain to synthesize enzymes that degrade the cell walls of the starchy endosperm is an important determinant of malt extract values. Cell walls act as barriers to the diffusion of starch- and protein-degrading enzymes during germination. Inadequate degradation of the cell wall will result in diminished degradation of starch and proteins, and therefore lower malt extract values (Fincher & Stone, 1993).

The extract is a mixture of soluble malt components, made up of mainly sugars and dextrin, and also nitrogenous compounds and minerals. As it is very laborious to analyze each component, the physical property, density, is analyzed. Density must then be converted to the weight of extract in solution, or Plato. The conversion is based on sucrose solutions because sucrose has a similar density to maltose, the main ingredient in extract (Anger *et al.*, 2009). A value of > 81% extract is required in South Africa (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009) and this property is determined according to the method in the Analytica EBC Methods Manual, Section 4.5.1 (European Brewery Convention, 1998).

6.2.2 Total nitrogen content

The total nitrogen content (TN) of malt and wort is directly influenced by the protein content of the barley it was obtained from (Bamforth & Barclay, 1993) and the presence of low nitrogen content in a barley sample indicates its potential to provide high extract malt (Foster *et al.*, 1967; Arends *et al.*, 1995; Eagles *et al.*, 1995; Molina-Cano *et al.*, 1997). Brewers demand the percentage nitrogen (dry basis) in malt to be 1.76% ± 0.06% (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009) which is determined in accordance with EBC methods 4.3.1 or 4.3.2 (European Brewery Convention, 1998).

6.2.3 Total soluble nitrogen

An important property for maltsters is the amount of nitrogenous material solubilised in wort; known as total soluble nitrogen (TSN) (Hough, 1991). The value is a direct comparison through reaction with formaldehyde of amino nitrogen contained in cold water extract with that found in wort after mashing (Pollock, 1962). Soluble nitrogenous compounds are a source of food for yeast during the brewing process and may determine the effectiveness of fermentation (Pollock, 1962). The extent to which endosperm degradation has taken place during malting will influence the amount of solubilised nitrogenous substances (Bamforth & Barclay, 1993). The TSN is measured on a Kjeltec Tecator according to EBC method 4.9.1 (European Brewery Convention, 1998) and a TSN value of

0.04% is required in South Africa (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

6.2.4 Kolbach Index

The Kolbach Index (KI) or soluble nitrogen ratio relates TSN to TN on a percentage basis. Brewers demand the KI to be sufficiently high to indicate protein modification has proceeded to a desired extent (Bamforth & Barclay, 1993). The KI thus indicates the level of malt modification (Hough, 1991; Kotze, 2009b) as it is a measure of the extent of protein conversion (Hough, 1991) and the higher the number, the more highly modified the malt. It also indicates how much protein is extractable and how much will remain in the grain; the KI tends to decrease as the TN increases (Hough, 1991) and should be between 40 and 45 (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). Malt with a too high KI may owe this to an effect of malting conditions or the degree to which its proteins were broken down rather than to an intrinsically high level of proteolytic enzymes (Pollock, 1962). KI is determined according to the method in the Analytica EBC Methods Manual, Section 4.3.1 (European Brewery Convention, 1998).

6.2.5 Free amino nitrogen

The yeast used in the brewing process requires a certain level of usable nitrogenous material for metabolic purposes. Nitrogenous materials such as amino acids are known as FAN (Bamforth & Barclay, 1993). FAN promotes yeast growth and is a determinant of the extent of fermentation by yeast (Bamforth & Barclay, 1993; Kotze, 2009b). High levels of FAN are undesirable as it influences the microbiological stability of the final beer product (Bamforth & Barclay, 1993). FAN in wort is measured with the Skalar segmented flow analysis method according to EBC method 4.10 (European Brewery Convention, 1998) and brewers demand the FAN value to be between 170 and 220 mg/L (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

A photometric analysis is carried out for the determination of FAN in wort. The principle of the analysis is based on the addition of ninhydrin, a colouring agent, to the sample. After boiling, parts of the ninhydrin are reduced by amino acids from the sample. In a subsequent reaction, non-reduced ninhydrin together with reduced ninhydrin and ammonia set free from amino acids form a coloured substance, which is measured photometrically at 570 nm, providing information about the amount of nitrogen derived from amino acids in the sample (Anger *et al.*, 2009).

6.2.6 Diastatic power

The ability of malt to convert starch to fermentable sugars is an important quality factor and is quantified by diastatic power (DP). Diastatic power reflects the combined activity of four starch reducing enzymes (α-amylase, β-amylase, limit-dextrinase and α-glucosidase) which degrade starch hydrolytically, to provide simpler, fermentable sugars and more complex unfermentable oligosaccharides (Duffus & Cochrane, 1993; Shewry & Darlington, 2002; Kotze, 2009b). The α-

amylase enzyme is responsible for the breakdown of starch to smaller oligosaccharides. β-amylase is the second most active enzyme after α-amylase (Shewry & Darlington, 2002). It attacks the reducing ends of starch, and a simple disaccharide, maltose, is liberated. The limit-dextrinase enzyme is important in the cleavage of 1-6 branches in amylopectin, producing more oligosaccharides for α-amylase to attack, while α-glucosidase acts on 1,4-alpha bonds (Duffus & Cochrane, 1993). The action of these enzymes on starch suspensions lead to decreases in viscosity and to the formation of the most important fermentable sugar, maltose (Pollock, 1962).

The determination of the DP in malt is a titration method. An extract is prepared from milled malt and the amount of maltose released from a standardized starch solution, under defined conditions, is measured by iodometry (Anger *et al.*, 2009). The DP measurement gives an overall measurement of β-amylase and α-amylase activity as determined from the release of reducing sugars in the buffered starch solution (Bamforth & Barclay, 1993). DP is measured with the Skalar flow injection method according to EBC method 4.12 (European Brewery Convention, 1998) and the DP value should be >300 Windisch Kolbach units (W.K). (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

6.2.7 Wort β-glucan content

β-glucans, the major component of the endosperm cell wall (Fincher & Stone, 1993), are unwanted in malting and brewing processes. High wort β-glucan levels indicate incomplete cell wall degradation and diminished mobilization of the starch-protein matrix. This results in lower malt extract values and lower nutrient availability for fermentative growth by yeast during brewing (Duffus & Cochrane, 1993). In addition, high wort β-glucan levels could contribute to negative beer characteristics such as beer haze or instability in shelf life. β-glucan levels in wort are measured with the Skalar segmented flow analysis method according to EBC method 8.13.2 (European Brewery Convention, 1998). High wort β-glucan content results in high wort viscosity, which in unwanted in the brewing process. A β-glucan value of <100 mg/L is required in South Africa (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

6.2.8 Wort viscosity

The viscosity of wort provides useful information about the degree of malt modification (Pollock, 1962). An aqueous solution of β-glucans is very viscous. β-glucans decrease rapidly during malting and is almost absent from the finished malt. Because of this fall in β-glucan quantity and the relationship between this polysaccharide and the cell walls of barley endosperm, viscosity is a measure of the breakdown of β-glucans during malting (Kotze, 2009b). Insufficiently degraded, high molecular weight β-glucans and arabinoxylans (also known as pentosans) originating from the cell walls of the endosperm contribute to high viscosity (Ullrich, 2002; Anger *et al.*, 2009) and therefore, the higher the wort viscosity, the lower the recovery of malt extract (Kotze, 2009b). Wort viscosity is measured in accordance with the EBC 8.4 method (European Brewery Convention,

1998). A maximum wort viscosity value of 1.55 cP is required for brewing in South Africa (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

6.2.9 Apparent attenuation limit

Attenuation is a term used to describe the limit to which fermentation proceeds and refers to the percentage of extract converted to alcohol during fermentation. The apparent attenuation limit (AAL) is therefore an indication of fermentability: the amount of alcohol that can be obtained from wort (Kotze, 2009b). For beer, a constant degree of attenuation is needed (Bamforth & Barclay, 1993). AAL is determined by small-scale laboratory fermentations under controlled conditions, using an excess amount of yeast. The specific gravity of the wort is measured before and after fermentation, and allows measurement of the extent to which the yeast is able to decrease the specific gravity of the wort (Bamforth & Barclay, 1993; Anger *et al*., 2009; Kotze, 2009b). This value is most relevant if the principal target is a maximum alcohol yield (Bamforth & Barclay, 1993). AAL is measured in accordance with EBC method 8.6.1 (European Brewery Convention, 1998) and an ideal value of >80% is demanded in industry (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009).

7. Quality evaluation

The problem facing breeders who are concerned with the improvement of malting barley is the accurate assessment of grain quality in breeding programmes. This requires a protocol involving a minimal number of individual tests which can be conducted efficiently on small amounts of grain. Such a method must be operable on the large numbers of samples which have to be examined in the early stages of the breeding programme and the tests conducted on the barley must be indicative of important malt characters, while any small-scale malting tests must provide an acceptably accurate guide to potential large-scale malting behaviour (Bell & Lupton, 1962). The earliest stage at which selection can be done is the F2 generation, when the criteria have to be based on the behaviour of individual plants. Field selection will reduce the number of plants needed to be examined on their grain characters, but the extent to which the visual characters of the grain from single plants can be used to act as guides to the potential of the material must be considered (Bell & Lupton, 1962).

Malting quality of a line is assessed by chemical analyses, micro-malting trials and finally full scale malting trials (Briggs *et al*., 1981a). Micro-malting techniques date back to as early as 1895, with small scale tests used by many maltsters through the ages (Meredith *et al*., 1962). Micro-malting probably originated due to the fact plant breeders wanted to obtain malting results with the simple apparatus at their disposal (Meredith *et al*., 1962). Various methods exist, each using different sample sizes (Whitmore & Sparrow, 1957; Meredith *et al*., 1962; Atkinson & Bendelow, 1976; Gothard *et al*., 1980) and attempt to obtain a reliable estimate of malt extract, enzymatic properties and wort quality (Meredith *et al*., 1962). Micro-malting remains a time consuming and

laborious method. It is also a destructive technique requiring larger samples (200 g) than are available in the earlier generations of breeding programmes, and can only be applied for quality evaluation at later stages in the programme, from year 8 onwards (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). If sample sizes are too small, more water is taken up during steeping which produces malt with higher moisture content. This leads to a lower malt yield and higher malt extract for small samples which does not allow accurate prediction of malting performance on a commercial scale (Henry & McLean, 1984).

The main problem is that breeding materials are limited in amount and are grown under one set of conditions in earlier generations (F1 to F4). When material has reached the stage of small-scale field trials, replicated samples become available, but such replication is also limited since it is generally confined to a single trial. As further selection and elimination proceeds, the field trials are usually conducted at more than one site in a year and a better picture can be obtained regarding the malting potential of selections and information can be accumulated on environmental adaptability and adaptation to particular conditions. Nonetheless, it is not until the final selections are under full-scale field trials that really comprehensive quality testing can be applied (Bell & Lupton, 1962; Kroonenberg, 1995).

This quality evaluation is expensive and requires large quantities of grain not available in early generation breeding lines. Therefore, breeders need a rapid, objective technique to be applied to small sample sizes, which would allow the prediction of malt quality properties from whole grain barley. This would ultimately allow for the elimination of poor malting lines in early stages of the breeding programme. At present, South African breeders use NIR spectroscopy in transmittance mode (800-1100 nm) for limited quality evaluation by predicting the nitrogen and moisture contents of whole grain barley, while also applying micro-malting tests to evaluate the malting potential of selected cultivars. SABM conduct micro-malting tests in order to determine which lines have acceptable malt properties to progress to the next phase of the breeding programme. Replicates of lines (grown on three different plots for each trial site) are bulked to save time and money (by reducing the number of samples to be tested) and an average value for these three samples is obtained (F. Smit, SABBI, Caledon, South Africa, Personal Communication, 2009). Sample bulking is not ideal, since it results in an average malt quality value for the three repetitions, and ignores the presence of sample variance within a single trial. With 100 g of seed available as early as the F5 generation, NIR could be implemented for quality testing, without the need for sample bulking, thus allowing quick evaluation of all samples.

8. Near infrared spectroscopy

8.1 Background

The NIR region was discovered in 1800 by Sir William Herschel, a musician who also discovered the planet Uranus (Pasquini, 2003). He was searching for the colour of glass that would pass the maximum amount of light with the least amount of heat, to be used in a telescope (Butler, 1983).

While experimenting with colours of light carrying the warmth of the sun (Osborne, 1981; Butler, 1983), he observed light was diffracted through a prism. He measured the temperature of these different colours. As the thermometers were moved from the blue to the red end of the rainbow, the temperature increased and continued increasing even past the visible region (Butler, 1983; Davies, 1998). He concluded the warmth of the sun was carried by waves that are not visible to the human eye and that there is energy beyond the red light. He gave these waves the term 'infrared' (IR), meaning 'beyond the red' (Osborne, 1981; Butler, 1983; Pasquini, 2003).

Although the NIR region was discovered before the mid-infrared (MIR) region, MIR was used by Coblentz to obtain the first absorbance spectra of pure substances. He observed compounds with similar chemical groupings, have characteristic absorption bands in the infrared and verified its usefulness for the identification of organic functional groups (Osborne, 1981; Pasquini, 2003). The NIR region was largely neglected mainly due to the fact that spectroscopists had difficulty interpreting the broad overlapping peaks of this region (Pasquini, 2003). The utility of NIR spectroscopy reflects the general availability of computers and development of chemometric techniques (mathematical techniques) (Davies, 1998).

The contributions of Karl Norris (named the 'First Fellow of Near Infrared Spectroscopy' by the NIR community (Davies, 1998) and generally regarded as 'The Father of NIR' (Butler, 1983)) have been instrumental to the everyday applicability of NIR spectroscopy. Norris, of the Beltsville United States Department of Agriculture (USDA) laboratory, recognized the potential of diffuse reflectance measurements in the NIR for the quantitative analysis of major components in agricultural commodities (Wetzel, 1983). Other important contributions were made by Phil Williams, Fred McClure and John Shenk (Davies, 1998).

8.2 Principles of NIR spectroscopy

NIR spectroscopy can be described as the interactions of NIR energy with matter (McClure & Tsuchikawa, 2007). The part of the electromagnetic spectrum, observed by the human eye, termed the visible region, extends from 400 to 750 nm, while the IR region is located from 2500 to 15 000 nm. The NIR region is situated between the IR and visible region (Osborne, 1981), from 750 to 2500 nm (Butler, 1983). NIR spectroscopy is a type of vibrational spectroscopy that employs photon energy in the range 2.65×10^{-19} to 7.96×10^{-20} J (Pasquini, 2003) and is based on absorption of electromagnetic radiation in the NIR wavelength range (Osborne, 2000).

Like radiation, NIR behaves as a wave with the properties of a simple harmonic motion. Chemical bonds between atoms in molecules are oscillators which vibrate constantly. This vibration is approximately a simple harmonic motion (Osborne, 1981; Osborne, 2000). When molecular vibrations occur at the same frequency as that of the radiation wave, a net transfer of energy from the radiation to the molecule will occur (Osborne, 2000). Vibrations can only occur at fixed frequencies and radiation is absorbed in discrete packets. A molecule can therefore only

absorb at specific fixed frequencies (Osborne, 1981). This energy transfer can be measured as a plot of energy versus wavelength and is called a spectrum (Osborne, 2000).

NIR spectra are composed of absorptions due to the overtones and combinations of fundamental stretching or bending vibrations (Davies, 1998; Pasquini, 2003). Each overtone band will be less intense than the preceding one, while combination bands arise by the interaction of two or more vibrations taking place simultaneously (Osborne, 2000). Spectra in the NIR region are the result of vibrations of light atoms with strong molecular bonds. If the atoms are heavy or the chemical bond between molecules is too weak, the vibrational frequency will be too low for its overtones to be detected in the NIR region (Wetzel, 1983). Molecules containing hydrogen atoms have a measurable NIR spectrum, allowing for the determination of a large number of chemical properties, as hydrogen is nearly universal. Chemical bonds containing hydrogen atoms attached to carbon, nitrogen or oxygen are predominantly observed, limiting the functional groups that are observable in the NIR region to more simple structures, common in most organic compounds (Wetzel, 1983; Davies, 1998; Osborne, 2000; Pasquini, 2003). Many constituents of foods absorb MIR wavelengths strongly and the weaker overtone and combination band absorptions of the NIR region enable spectra to be measured with greater ease. The absorption bands due to constituents such as protein, oil and moisture are strong enough in the NIR to be measured accurately (Osborne, 1981).

Correlation charts showing where absorption bands of O-H, C-H, N-H and S-H bonds of certain compounds are located in the NIR region can be used for qualitative analysis (Osborne *et al.*, 1993; Siesler *et al.*, 2002). Determination of the concentrations of constituents such as water, protein, fat and carbohydrate using absorption spectroscopy is also possible (Osborne, 2000). There is, for example, a prominent peak at 1930 nm in the spectrum of water and measurement of the magnitude of this peak can be related to the amount of water present in a sample (Osborne, 1981).

NIR spectroscopy requires calibration against a reference method for the constituent of interest (Osborne, 2000). The application of NIR spectroscopy is based on the empirical relationship between reference data obtained by conventional analytical methods and spectral data measured with a spectrometer (Pasquini, 2003). When computing a set of calibration constants in NIR reflectance technology, the reference values are the dependent variables (y) and the optical data (log 1/R or absorbance values) are the independent variables (x) (Williams, 2001).

8.3 Measurement modes

Light directed onto a sample may be transmitted or reflected and NIR instruments can operate in reflectance or transmittance mode. In reflectance mode, the light source and the detector are on the same side of the sample (above or below). As light illuminates the surface of the sample, only some of the light is absorbed and the remainder is diffusely reflected from the surface. The study of the reflected light can be used to measure the amount of light absorbed (Osborne, 1981; Kawano,

2002). In this case, the sample should be opaque, for example a powdered sample. Usually, light cannot reach a deeper position in a sample due to high absorption or multiple scattering. If samples have sufficient thickness, the optical sample thickness should be regarded as infinite and attention may only be paid to the absorption coefficient. This is very useful for analyzing NIR spectra of powdered and solid samples and a sample of more than 1 cm depth is recommended (Tsuchikawa, 2007).

In transmittance mode, the light from the light source passes through the sample and is received by the detector on the other side of the sample (Kawano, 2002). This mode is widely applicable for liquids without scattering or in low scattering conditions where a cuvette is used (Tsuchikawa, 2007).

Development of NIR spectroscopy as a unique analytical technique began when Karl Norris proposed a spectral measurement could be obtained by analysing the portion of radiation diffusely reflected by solid samples instead of the weaker signal of transmittance. Today, diffuse reflectance is widely employed in the NIR spectral region (Pasquini, 2003).

8.4 Advantages and disadvantages of NIR spectroscopy

NIR spectroscopy is advantageous since it offers the potential to conduct rapid tests on small samples of ground grain or non-destructively on whole grain (Woodcock *et al.*, 2008). It is particularly known for its accuracy, simplicity and safety (Osborne, 1981). Reproducibility is equal to and at times better than reference measurements (Williams, 2007). It allows for the simultaneous measurement of multiple quality properties (Sissons *et al.*, 2006) since it is not necessary to repeat scans for each constituent (McClure & Tsuchikawa, 2007). NIR spectroscopy is fast, taking no more than one minute per sample, it is non-invasive and requires no sample preparation (Pasquini, 2003). No sample dilution is needed as absorptions in the NIR region are much weaker than in the IR region (Davies, 1998).

A major disadvantage of NIR is the dependence on less precise reference methods (Osborne, 2000). NIR instrumentation must be calibrated by scanning a set of samples with known qualitative or quantitative properties, involving expensive and complicated reference methods that require highly skilled personnel. Modern day calibrations are dependent on sophisticated chemometric techniques, which also require the expertise of trained personnel (McClure & Tsuchikawa, 2007). The most apparent disadvantage has always been that separate calibrations are needed for each constituent. There is also the need to monitor accuracy and precision regularly and it is expensive to purchase NIR instruments (Williams, 2007).

8.5 Near infrared instrumentation

Successful use of NIR spectroscopy depends on determining the most appropriate instrument for the application and three different types are available (Osborne *et al.*, 1993; Osborne, 2000). Instrument selection must be considered for the end application be it for research, in-line

monitoring or laboratory/in-field applications (Pasquini, 2003). Ideally, the instrument should be able to accommodate at least 100 g of sample, to minimize sampling error (Williams, 2001).

Grating monochromators are the most versatile instruments and are used to measure the full visible and NIR spectrum in transmittance or reflectance mode. Such equipment is used when a wide range of different applications is required or when spectral information from a wide range of wavelengths is necessary for the development of an accurate and stable calibration (Osborne, 2000).

Interferometers are modulators that do not produce angular dispersion and fall into two groups, double-beam and multiple beams (Osborne *et al*., 1993). Fourier-transform (FT) instruments are based on the use of interferometers and FT to recover the intensities of individual wavelengths in the NIR region and combine the best characteristics in terms of wavelength precision and accuracy, high signal-to-noise ratio (S/N) and scan speed (Pasquini, 2003). An interferometer offers excellent resolution and wavelength reproduction in comparison to a grating monochromator (Wetzel, 2001).

Filter instruments are the simplest and cheapest NIR instruments and are based on a limited number (between six and twenty) of interference filters. These filters are chosen as wavelength selectors to represent the absorptions used for the most popular applications, e.g. protein, moisture and oil in agricultural samples (Osborne, 2000; Pasquini, 2003). Filter instruments are designed for a limited range of routine analysis, either in the laboratory or on-line (Osborne, 2000) and remain economical alternatives for NIR analysis (McClure, 2003).

The most frequently employed detectors for the NIR spectral region are based on silicon, lead sulphide (PbS) and indium gallium arsenide (InGaAs) photoconductive materials (Osborne, 2000; Pasquini, 2003). A silicon detector covers the range 400 to 1100 nm, an InGaAs detector covers the range 800 to1700 nm and a PbS detector covers the range 1100 to 2500 nm (Osborne, 2000). High powered radiation sources such as a tungsten coil or halogen lamp are employed by the majority of manufacturers and can impart a very high S/N for NIR measurements, which compensates for lower intensities of NIR absorption bands (Pasquini, 2003).

Modern-day NIR instruments are continually changing as additional features and flexibilities are added with every new instrument. Portable and hand held instruments remain a keen interest in this emerging line of work (McClure, 2003).

8.6 Calibration development

An NIR calibration model is developed by selecting a set of reference or calibration samples with known analyzed concentration (obtained by reference methods). The set of calibration samples should contain the range of chemical and physical variations expected in the samples the calibration model will be applied to. The purpose of this calibration experiment is to establish a mathematical relationship between the NIR spectrum and physical/chemical properties determined by reference methods. The accuracy of this mathematical relationship may be tested using the NIR

spectra of independent samples (validation/test set) to predict the chemical or physical properties of interest (Bokobza, 1998; Cen & He, 2007); this is known as test set validation. It is imperative that samples contain all possible sources of variation found in the independent test set due to physical or chemical presentations. A good knowledge of the sample set enables the optimal use of NIR spectroscopy for quantitative purposes (Pasquini, 2003). Sample sets should be set up so the ratio of calibration to validation samples is 3:1. If calibration sets are too small, the calibration may be sample sensitive and analysis of fresh batches of samples may show significant differences in accuracy. In a perfect world, the calibration and validation sets should not be related to one another, but both should embrace the same variation dimensions (Williams, 2001).

If the available sample set consists of up to only 60 samples, the calibration is best evaluated with cross-validation. Cross-validation involves the division of the sample population into blocks consisting of one (leave-one-out cross-validation) or more (segmented cross-validation) samples. For both cross-validation procedures, the same steps are followed; samples are eliminated one at a time, or one block at a time, from the 'training set' and a calibration model calculated using the remaining samples. This calibration model is used to predict the property of interest for the removed samples. The eliminated samples are put back into the sample set, another block is removed and the calculation procedure is repeated until all blocks have been removed. This procedure suffers from criticism since samples used for validation are selected from the original sample set, whereas ideally samples used in the evaluation should be obtained independently (Williams, 2001).

The number of samples included in the calibration sample set is important. Factors affecting the samples such as environment and seasonal variations must be taken into consideration, and all factors must be represented in the sample set. Some samples may be classified as outliers; samples that do not belong to the majority of the sample population. These samples may differ due to spectral characteristics or an error in its reference values obtained by conventional methods (Pasquini, 2003). Accuracy of the reference method is extremely important, as errors in the reference methods will be inherent in the calibrations developed. High quality calibrations can only be developed if reference methods are precise and accurate (Edney *et al*., 1994; Williams, 2001).

The Kennard and Stone algorithm can be applied for selection of a representative test set (or sub set) from a population of samples. This algorithm selects an object which is closest to the data mean and adds it to the subset; calculates the dissimilarity between the object in the subset and the remaining samples and the object which is most dissimilar to the one already included to the subset is added to the subset. This is repeated until the desired number of objects in the subset is reached (Daszykowski *et al*., 2002).

The jack-knife based method (also referred to as 'uncertainty testing' (Davies, 2001)) is based on significance testing of model parameters, applied to regression coefficients, thereby eliminating useless or unreliable variables in order to simplify the final model and make it more reliable. The approximate uncertainty variance of PLS regression coefficients is estimated by significance tests,

where a t-test is performed for each element in the regression coefficient relative to the square root of its estimated uncertainty variance, giving the significance level for each parameter. The uncertainties for the regression coefficients are estimated for a specific number of components, preferably the optimum number. The useless variables are removed as long as the root mean square error of performance (RMSEP) decreases. This method has several desired properties as it is computationally simple, has significance tests of model parameters and is robust toward cross-validation schemes (Westad & Martens, 2000; Esbensen, 2002). The general rule is that if a model with fewer variables is as good as or better with respect to predictability as the full model, the simpler model is preferred. There are a large number of applications that utilize only two or three wavelengths in routine prediction and these applications have shown that the full PLS model is sometimes inferior to a model based on a relatively small number of variables; probably due to redundancy and large amount of noisy, irrelevant variables in the NIR spectra. Results have shown variable selection, based on jack-knife estimates, is a fast and reliable method with low risk of over fitting (Esbensen, 2002).

8.6.1 Chemometrics and multivariate calibration methods

The practice of extracting chemical and physical information from relevant NIR spectra with the application of mathematical and statistical tools is known as chemometrics (Wold, 1995; Bokobza, 1998; Pasquini, 2003). It is used to relate the physical or chemical properties of a sample to the absorption of radiation in the NIR wavelength range. NIR spectral data contain a great deal of physical and chemical information. This cannot always be extracted, seeing as the NIR spectra consist of a number of overlapping bands which cannot be interpreted as easily as MIR spectra, which exhibit sharp and narrow peaks. Statistical methods have allowed for the extraction of qualitative as well as quantitative information from these complex NIR spectra (Bokobza, 1998).

NIR spectral data can be pre-treated before it is used for quantitative and qualitative analysis. Pre-treatment is used to overcome problems associated with radiation scattering by a solid sample (Beebe *et al.*, 1998). Mathematical pre-processing techniques allow for the extraction of relevant information from the raw spectral data, which might contain defects, prior to analysis (Bokobza, 1998). Pre-treatment can be used for several purposes, i.e. removal of random noise; reduction of the physical effect of sample variation in scatter caused by particle size differences; and enhancement of weak absorption bands. The best pre-treatment is not known beforehand and the analyst must manually search for the technique that delivers the best results (Delwiche & Reeves, 2004).

Pre-treatment techniques include derivatives (Massart *et al.*, 1988; Næs *et al.*, 2002), normalisation (Massart *et al.*, 1988; Næs *et al.*, 2002), multiplicative scatter correction (MSC) (Geladi *et al.*, 1985), standard normal variate (SNV) (Barnes *et al.*, 1989) as well as smoothing techniques such as the moving average method (Savitzky & Golay, 1964).

Derivative pre-treatment is easy to perform and reduces scatter effects in continuous spectra. The second derivative is particularly useful if the absorbing species present sharp spectral bands (Næs *et al*., 2002). Normalisation involves changing spectra so that resultant spectra have more features in common or unwanted sources of variability are suppressed, which helps the visual understanding of the spectra (Hruschka, 2001). MSC is also a normalisation procedure which separates chemical light absorption from physical light scatter (Geladi *et al*., 1985). SNV transforms spectral data by subtracting the mean of the spectra from each spectrum, and scaling all spectra by the standard deviation of the spectrum. SNV removes multiplicative interferences of scatter and particle size (Barnes *et al*., 1989). The Savitzky-Golay moving average reduces the effect of noise on a spectrum by removing meaningless variations in absorbance (Savitzky & Golay, 1964).

Exploratory analysis such as principal component analysis (PCA) can provide insight into the variation in the data. PCA can be applied to spectral data to give indications of relationships between samples during the early stages of data analysis (Cowe & McNicol, 1985).

Quantitative chemometric techniques such as regression methods are commonly used in calibration development. Partial least square (PLS) regression establishes a linear relationship between spectral data and the property value that needs to be determined (Wold *et al*., 2001). The result is a calibration equation from which the property of interest can be predicted. The equation is evaluated by statistics which define the difference between the actual and predicted values (Osborne, 2000).

8.6.2 Statistical evaluation

Statistics are used to evaluate the efficiency of NIR calibrations (Williams, 2001) and various terms are important in understanding the performance of a calibration model. Important statistics that should be included when reporting NIR results are shown in **Table 2.1**. This includes statistics of calibration as well as statistics of validation.

For calibration statistics, it is important to include the property evaluated as well as its units. The standard error of laboratory (SEL) is a good indication of the error of the reference data. The number of samples evaluated as well as the number of outliers that were removed should be reported. Minimum, mean and maximum values as well as the standard deviation (SD) of the reference data should be included (Dardenne, 2010). The SD expresses the variance in the reference data (Williams, 2001). The coefficient of determination (R^2 for calibration and cross-validation) shows the proportion of variance in the reference data explained by the variance in spectral data (Williams, 2001). The standard error of calibration (SEC), the standard error of cross-validation (SECV) and root mean square error of cross validation (RMSECV) should be reported. NIR repeatability, and the Ratio of standard error of Prediction Validation to standard Deviation (RPD) for calibration (RPD_C) and cross-validation (RPD_{CV}) should be included (Dardenne, 2010). The RPD is calculated by dividing the SD of the reference values used in prediction by the

standard error of performance (SEP) and allows for the evaluation of the SEP in terms of the SD of the reference data. This gives an indication of the efficiency of a calibration model (Williams, 2001). The number of cross-validation segments should also be included. Lastly the wavelength range, all pre-treatments used as well as the regression method used should be included.

Validation statistics include the same elements as calibration statistics in **Table 2.1** until reference SD, and is followed by r^2 (coefficient of determination for validation), the root mean square error of prediction (RMSEP) and the SEP (Dardenne, 2010). The SEP is the square root of the average of the sum of squares between NIR predicted and reference values, and is calculated from the predictions made in test set validation. The SEP should be as small as possible while an r^2 value as close as possible to one is desired (Williams, 2001). The RMSEP gives a measure of the efficiency of a calibration and includes bias error. The bias measures the overall accuracy of the calibration as it indicates the difference between the reference and NIR reflectance data and should be as close to zero as possible (Williams, 2001). The residual standard deviation (RSD) indicates the error after bias and slope correction. NIR repeatability is again reported, along with the bias, intercept and the slope of the regression (Dardenne, 2010).

Key formulae used to calculate statistical parameters are summarized in **Table 2.2**. Guidelines for the interpretation of r^2 are summarized in **Table 2.3** while guidelines for the interpretation of the RPD are set out in **Table 2.4**.

Table 2.1 Important statistics to evaluate the efficiency of a calibration (Dardenne, 2010)

Statistics of calibration	Statistics of validation
Parameter	Parameter
Units	Units
SEL – Reproducibility	N
N	Outliers
Outliers	Min
Min	Mean
Mean	Max
Max	SD
SD	r^2
SEC	RMSEP
R^2	SEP
SECV	RSD
R^2CV	NIR repeatability
NIR repeatability	Bias
Number of terms	Intercept
RPD_C	
RPD_{CV}	
Segments (LOO)	
Wavelength range	
Pre-treatments	
Regression method	

SEL=standard error of laboratory; N=number of samples; Min=minimum; Max=maximum; SD=standard deviation; SEC=standard error of calibration; R^2=coefficient of determination for calibration; r^2=coefficient of determination for validation; RMSEP=root mean square error of prediction; SEP=standard error of prediction; SECV=standard error of cross-validation; R^2CV=coefficient of determination for cross-validation; RSD=residual standard deviation; RPD_C= Ratio of standard error of Prediction Validation to standard Deviation for calibration; RPD_{CV}= Ratio of standard error of Prediction Validation to standard Deviation for cross-validation; LOO=leave one out (cross-validation)

Table 2.2 Equations for statistical calculations

Statistic	Equation	Recommendations
SD[a]	$\sqrt{\sum y^2 - \frac{\left[\frac{\left(\sum y\right)^2}{n}\right]}{n-1}}$	
SEL[b]	$\sqrt{\frac{\sum\left(y_1 - y_2\right)^2}{2n}}$	As small as possible
SEP[c] / SECV[d]	$\sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i - BIAS\right)^2}{n-1}}$	As small as possible or close as possible to SEL value
BIAS[e]	$\frac{1}{n}\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)$	As close to zero as possible
r[f]	$\frac{\sum_{i=1}^{n_s}\left(\hat{y}_i - y_i\right)^2}{\sqrt{\sum_{i=1}^{n_s}\left(y_i - \hat{y}\right)^2}}$	See **Table 2.3**
RPD[g]	$\frac{SD_{\hat{y}}}{SEP}$ or $\frac{SD_{\hat{y}}}{SECV}$	See **Table 2.4**

[a] Standard deviation

[b] Standard error of laboratory

[c] Standard error of prediction

[d] Standard error of cross validation

[e] Bias of the validation set

[f] Coefficient of correlation

[g] Ratio of standard error of performance to standard deviation

y = reference value

$\hat{y}$ = predicted value

y_i = reference value for the i^{th} sample

$\hat{y}_i$ = NIR predicted values for the i^{th} sample

y_1 and y_2 = duplicate reference values

n = number of samples

t = number of terms in the model

Table 2.3 Guidelines for the interpretation of r^2 (Williams, 2001)

Coefficient of determination	Interpretation
Up to 0.25	Not usable
0.26 - 0.49	Poor correlation
0.50 - 0.64*	Acceptable for rough screening
0.66 - 0.81	Can be used for screening and approximate calibrations
0.83 - 0.90	Usable with caution
0.92 - 0.96	Usable in most applications and quality assurance
0.98+	Can be used in any application

* Due to rounding off, no values of 0.65, 0.82, etc. are included in this table

Table 2.4 Guidelines for the interpretation of the RPD (Williams, 2001)

RPD	Classification	Application
0.0 – 2.3	Very poor	Not recommended
2.4 – 3.0	Poor	Rough screening
3.1 – 4.9	Fair	Screening
5.0 – 6.4	Good	Quality control
6.5 – 8.0	Very good	Process control
8.1+	Excellent	Any application

RPD=Ratio of standard error of Prediction Validation to standard Deviation

9. Prediction of malting barley quality with NIR spectroscopy

Studies regarding the prediction of barley quality as well as malt quality characteristics with NIR spectroscopy in reflectance mode, both on whole grain and ground barley and malt, as well as on wort, have delivered suitable prediction models in a number of cases. Studies performed with instruments only operating in transmittance mode include the determination of nitrogen and moisture contents on whole grain barley (Williams *et al.*, 1985; Angelino, 1996) as well as on malt (Angelino, 1996) and the determination of FAN, extract and fermentability on wort (Halsey, 1986).

9.1 Barley quality

9.1.1 Whole grain barley

The results of previous analyses of agronomic quality properties on whole grain barley are summarized in **Table 2.5**. Prediction models for nitrogen content were developed by Halsey (1987) and the relatively low SEP and r^2 of 0.71 (Halsey, 1987) proved this calibration to be acceptable for use as a rough screening method. A study by Edney *et al.* (1994) delivered excellent nitrogen content prediction models that could be used in most applications, as indicated by the high r^2 (0.94) and the low SEP (0.31%) values. The very high RPD of 4 indicated the calibration would be acceptable for screening purposes (Edney *et al.*, 1994). Prediction models for nitrogen content

have also been developed with the use of cross-validation and obtained a relatively good R^2 of 0.83 (Li *et al.*, 1995). The nitrogen content of whole grain barley was also determined with cross-validation by Sohn *et al.* (2008), which delivered an excellent correlation (R^2 = 0.95) but the model could only be used for rough screening purposes, as indicated by the low RPD of 2.67 (Sohn *et al.*, 2008).

Prediction models for the determination of moisture content from whole grain barley indicated very good predictions could be achieved with this model (R^2 = 0.94) and it could be used in most applications, although no SEP was reported (Downey, 1985). Halsey (1987) developed a model for prediction of moisture content and obtained an excellent r^2 of 0.96 and low SEP of 0.15% (Halsey, 1987) and this calibration would be acceptable for use in most applications. Cross-validation was also applied for the prediction of moisture content and the calibrations would only be acceptable for rough screening due to the average R^2 value of 0.76 (Li *et al.*, 1995). The moisture content of whole grain barley was also determined by cross-validation by Sohn *et al.* (2008) and delivered excellent calibrations (R^2 = 0.96) that could be used in most applications. The RPD was relatively low (3.73) which indicated that this model would be acceptable for screening (Sohn *et al.*, 2008).

NIR measurements have been used to predict kernel plumpness from whole grain barley where the high SEP of 11.5 was attributed to a poor range of the percentage plump kernels included in the study, which could also account for the low RPD of 2.4 (Edney *et al.*, 1994). This calibration would therefore only be acceptable for rough screening purposes (Williams, 2001).

9.1.2 Ground barley

Studies on ground barley have focused on the prediction of nitrogen content and moisture content (**Table 2.6**). A very good calibration model for nitrogen content was developed where the relationship between NIR and predicted values was very close (r^2 = 0.92), confirming reasonably precise estimates can be made using NIR spectroscopy (Gill *et al.*, 1979) and the calibration would be acceptable for use in most applications. Researchers were able to develop excellent calibrations (r^2 = 0.99) for prediction of the nitrogen content of ground barley and the accuracy of the model was more than adequate for quality prediction in a barley breeding programme (Henry, 1985c). Good calibrations that were acceptable for use in most applications (r^2 = 0.92), were also developed for prediction of nitrogen content from ground barley (Tragoonrung *et al.*, 1990).

With the use of wavelength selection, excellent calibrations were developed for prediction of the moisture content of ground barley (Henry, 1985c) and these models could be used for prediction in any application (r^2 = 0.99). Downey reported no SEP but cross-validation delivered an excellent calibration with a very high R^2 of 0.98 for moisture (Downey, 1985).

9.2. Malt quality

9.2.1 Whole grain barley

The results for the prediction of malt quality properties from whole grain barley in various studies are shown in **Table 2.7**. An attempt to predict wort β-glucan levels from NIR spectra of whole grain barley delivered extremely poor results (r^2 = 0.25) and the model was not usable for prediction (Black & Panozzo, 2001). This was possibly due to the complex nature of the constituent and the proteins and starches in unmalted barley that were not yet modified by the action of enzymes during malting. Prediction results for β-glucan content of raw barley were suitable for screening and could be used for classification of barley into low and high groups. It was suspected the narrow range in reference values could have been the cause of this poor calibration (Sohn *et al.*, 2008).

Researchers developed extract calibrations that seemed promising in terms of predicting the extract potential of barley; the calibration could be used with caution for prediction purposes (r^2 = 0.85). It was found that an accurate calibration developed for malt might give accurate results but was not suitable for barley. Barley is much harder than malt and after the malting process, protein will be more loosely bound and the complex structure of the grain will be changed, allowing NIR radiation to penetrate more deeply into malt than barley (Halsey, 1987). Good calibrations were obtained for predicting extract from whole grain unmalted barley by Li *et al*. (1995) (R^2 = 0.75) and Black & Panozzo (2001) (r^2 = 0.78) that proved to be acceptable for screening purposes.

Acceptable calibrations were obtained for predicting DP from whole grain barley (r^2 = 0.59) but a high SECV of 30 W.K. was obtained. This parameter is mainly controlled by complex interactions of barley endosperm substrates and enzymes during malting (Li *et al.*, 1995) which may be the reason for these poor results. A very poor model for the prediction of DP was also reported (Black & Panozzo, 2001) but the NIR calibration was not usable in any application (r^2 = 0.37, SEP = 57 W.K.). This poor calibration can be attributed to the inability of the NIR method to account for the development of enzymes during malting and the extent of endosperm modification as a result thereof (Henry, 1985b).

The properties FAN and TSN were not predicted well from whole grain barley (Black & Panozzo, 2001) and NIR calibrations were unacceptable for use in prediction (Williams, 2001).

Acceptable calibrations were obtained for predicting wort viscosity with cross-validation (R^2 = 0.62, SECV = 0.02) and could be used for rough screening in breeding programmes (Li *et al.*, 1995).

9.2.2 Ground barley

Table 2.8 summarizes the results obtained from NIR spectroscopy studies conducted on ground barley for the prediction of malt quality. The coefficient of determination for the prediction of β-glucan content from ground barley (r^2 = 0.76) showed a good relationship between predicted and reference values. It was concluded the calibration could be used in assessment of malt quality properties (Allison *et al.*, 1978) and since it has a relatively high r^2 it could be used for screening

purposes. Wavelength selection of 3 and 6 wavelengths delivered calibrations for β-glucans (Henry, 1985c) that were acceptable for screening purposes (r^2 = 0.77). Good prediction models were obtained for prediction of β-glucan levels from ground barley, with a relatively high r^2 of 0.77 and low SEP of 1.13% (Szczodrak *et al.*, 1992) which indicated the model was adequate for rough screening.

Morgan and Gothard (1979) developed calibration models for winter barley for prediction of extract with a very low r^2 value of 0.49. Since the method only provides an indication of extract and cannot be expected to be very accurate (it employs raw grain which does not account for enzyme activity), it was concluded the method could not be used as a screening test in early generation selections (Morgan & Gothard, 1979). Another model developed for extract delivered very good results (r^2 = 0.96) but a too narrow range in malt values (74.1 – 79.2%) had an effect on the predictions (McGuire, 1982). However, the high r^2 value indicated the calibration could be used in most applications. The selection of three wavelengths delivered a good model for extract (r^2 = 0.88) that is usable with caution in most applications. The researchers also stated that enzyme activity during malting influences the malt extract and limits the accuracy of any NIR prediction based on ground barley (Henry, 1985c). A model acceptable for rough screening (r^2 = 0.77) was also developed for extract (Tragoonrung *et al.*, 1990).

Viscosity was predicted from ground barley (Allison *et al.*, 1978) and the results (r^2 = 0.65, SEP = 0.60 cP) proved the model to be acceptable for use in rough screening.

9.2.3 Whole grain malt

Studies conducted on whole grain malt for the NIR prediction of malt quality are summarized in **Table 2.9**. A study by Black and Panozzo (2001) included the properties extract, DP, β-glucans, FAN and TSN. A calibration acceptable for screening purposes was obtained for extract (r^2 = 0.76, SEP = 1 %) while a calibration for DP (r^2 = 0.54, SEP = 54 W.K.) showed promise for use in rough screening during early stages in breeding programmes. Relatively high r^2 values were obtained for wort β-glucan content (r^2 = 0.51), FAN (r^2 = 0.63) and TSN (r^2 = 0.53) and it was concluded that these calibrations were acceptable for rough screening of early generation breeding lines (Black & Panozzo, 2001).

9.2.4 Ground malt

Results obtained for the prediction of moisture, nitrogen and extract from ground malt are summarized in **Table 2.10**. Prediction models based on test set validation for moisture delivered an excellent model with r^2 = 0.98 (Henry, 1985a), which allows for use in any application. A study for prediction of malt moisture content delivered calibrations that were considered to be satisfactory for predicting these values when considering the low RMSEP and SEP values of 0.10% and the excellent correlations obtained (r^2 = 0.98) between predicted and reference values (Marte *et al.*, 2009).

Marte *et al.* (2009) also predicted malt nitrogen content and the calibration was considered to be satisfactory for prediction due to the low SEP value of 0.04% (Marte *et al.*, 2009) and the model was usable with caution in most applications (r^2 = 0.85).

Models for the prediction of extract from ground malt delivered good results (r^2 = 0.85) and these models could be used for most applications, but with caution (Henry, 1985a).

9.2.5 Wort

Ratcliffe and Panozzo (1999) predicted the malting properties extract, FAN and TSN from wort by selecting four wavelengths for each trait (selected through trial and error) and the results are shown in **Table 2.11**.

The prediction models for extract delivered very good results with an r^2 of 0.88 and could therefore be used for most applications. The TSN (r^2 = 0.80) and FAN (r^2 = 0.73) calibrations would be acceptable for rough screening purposes although the prediction model for FAN had a relatively high SEP of 15 mg/L. This error was close to standard method errors and researchers concluded that the FAN calibration could be used for identification of lines with unsatisfactory quality (Ratcliffe & Panozzo, 1999).

Table 2.5 Results obtained for NIR prediction of nitrogen content, moisture content and plumpness of whole grain barley

Parameter	Calibration set					Validation set						Wavelengths (nm)	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
Nitrogen content (%)	1.27-2.15	0.218	0.77	-	-	1.39-2.06	0.18	0.71	0.09	-	-	1692, 2152	2^{nd} der	Halsey (1987)
	9.4-15.5	1.26	-	-	-	9.6-15.3	1.25	0.94	0.31	-	4.0	-	2^{nd} der	Edney *et al.* (1994)
		0.07	0.83	0.26	-	-	-	-	-	-	-	-	-	Li *et al.* (1995)
	6.81-12.22	1.11	0.95	-	0.35	7.26-12.09	1.04		-	0.39	2.67	-	1^{st} der, MSC, MC	Sohn *et al.* (2008)
Moisture content (%)	13.6-20.0	0.38	0.94	-	-	-	-	-	-	-	-	1940	-	Downey (1985)
	11.0-14.3	0.851	0.96	-	-	11.2-14.2	0.70	0.96	0.15	-	-	2018	1^{st} der	Halsey (1987)
	-	0.19	0.76	0.26	-	-	-	-	-	-	-	-	-	Li *et al.* (1995)
	9.64-18.45	1.52	0.96	-	0.30	9.76-17.83	1.49	-	-	0.40	3.73	-	1^{st} der, MSC, MC	Sohn *et al.* (2008)
Plumpness (%)	4.2-96.5	21.5	-	-	-	4.7-94.5	27.2	0.83	11.5	-	2.4	1140, 1180, 1200	2^{nd} der	Edney *et al.* (1994)

SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; MSC=multiplicative scatter correction; 2^{nd} der=second derivative; 1^{st} der=first derivative; MC=mean centering

Table 2.6 Results obtained for NIR prediction of nitrogen content and moisture content from ground barley

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
Nitrogen content (%)	1.35-2.90	-	-	-	-	-	-	0.92	0.33	-	-	-	-	Gill *et al.* (1979)
	1.11-2.63	0.40	-	-	-	-	-	0.99	-	0.10	-	-	1st der	Henry (1985c)
	8.9-14.8	-	-	-	-	-	-	0.92	0.42	0.18	-	3 to 6 wavelengths	-	Tragoonrung *et al.* (1990)
Moisture content (%)	13.4-25.9	0.38	0.98	-	-	-	-	-	-	-	-	-	-	Downey (1985)
	5.9-16.8	3.17						0.99	-	0.13		3 wavelengths		Henry (1985c)

SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; 1st der=first derivative

Table 2.7 Results obtained for NIR prediction of β-glucans, extract, DP, FAN, TSN and wort viscosity from whole grain barley

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
β-glucan content (mg/L)	0-1089	-	0.77	-	-	0-760	-	0.25	240	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
β-glucan content (%)	2.33-5.76	0.66	-	-	0.52	2.66-5.66	0.63	0.80	-	0.43	1.47	-	2nd der, MSC, MC	Sohn *et al.* (2008)
Extract (l°/kg)	279-316	8.69	0.85	-	-	280-316	8.9	0.85	3.2	-	-	1686/1914, 2352	2nd der	Halsey (1987)
Extract (%)	-	0.21	0.75	0.43	-	-	-	-	-	-	-	-	-	Li *et al.* (1995)
	77-87	-	0.87	-	-	76-81	-	0.78	1.1	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
DP (W.K.)	-	33.0	0.59	30.0	-	-	-	-	-	-	-	-	-	Li *et al.* (1995)
	179-549	-	0.57	-	-	225-545	-	0.39	57	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
FAN (mg/L)	92-228	-	0.54	-	-	116-239	-	0.10	31	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
TSN (%)	4-6	-	0.60	0.2	-	4-6	-	0.01	0.5	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
Wort viscosity(cP)	-	0.01	0.62	0.02	-	-	-	-	-	-	-	-	-	Li *et al.* (1995)

DP=diastatic power; W.K.=Windisch Kolbach units; FAN=free amino nitrogen; TSN=total soluble nitrogen; cP=centipoises; SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate and de-trending; 2nd der=second derivative; MSC=multiplicative scatter correction; MC=mean centering

Table 2.8 Results obtained for NIR prediction of β-glucans and extract from ground barley

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
β-glucan content (%)	-	-	-	-	-	-	-	0.76	0.22	-	-	-	-	Allison *et al.* (1978)
	2.90-5.16	0.53	-	-	-	-	-	0.77	-	0.32	-	3 wavelengths	-	Henry (1985c)
	2.99-9.51	-	0.85	0.677	-	3.67-9.34	-	0.77	1.13	0.74	-	2234, 2374, 2500	-	Szczodrak *et al.* (1992)
Extract (%)	-	-	-	-	-	-	-	0.49	1.65	1.66	-	-	-	Morgan & Gothard (1979)
	74.1-79.2	-	0.90	-	-	-	-	0.96	-	-	-	-	-	McGuire (1982)
	46.9-62.2	3.77	-	-	-	-	-	0.88	-	2.29	-	3 wavelengths	-	Henry (1985c)
	71.3-85.1	-	-	-	-	-	-	0.77	1.33	1.69	-	6 wavelengths	-	Tragoonrung *et al.* (1990)
Wort viscosity (cP)	-	-	-	-	-	-	-	0.65	0.6	-	-	-	-	Allison *et al.* (1978)

cP=centipoises; SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; cP=centipoises

Table 2.9 Results obtained for NIR prediction of extract, DP, β-glucans, FAN and TSN from whole grain malt

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
Extract (%)	77-87	-	0.89	-	-	76-81	-	0.76	1.0	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
DP (W.K.)	179-549	-	0.75	-	-	225-545	-	0.54	54	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
β-glucan content (mg/L)	0-1089	-	0.83	-	-	0-760	-	0.51	165	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
FAN (mg/L)	92-228	-	0.89	-	-	116-239	-	0.63	17	-	-	-	SNV, 2nd der	Black & Panozzo (2001)
TSN (%)	4-6	-	0.79	-	-	4-6	-	0.53	0.3	-	-	-	SNV, 2nd der	Black & Panozzo (2001)

DP=diastatic power; W.K.=Windisch Kolbach units; FAN=free amino nitrogen; TSN=total soluble nitrogen; SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate and de-trending; 2nd der=second derivative

Table 2.10 Results obtained for NIR prediction of moisture content, nitrogen content and extract from ground malt

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
Moisture content (%)	-	-	-	-	-	-	-	0.98	-	0.26	-	1352,1982,2206	-	Henry (1985a)
	-	-	0.97	-	0.097	-	-	0.98	0.10	-	-	-	Constant offset elimination	Marte *et al.* (2009)
	-	-	-	-	-	-	-	0.98	-	0.10	-	-	-	Marte *et al.* (2009)
Nitrogen (%)	-	-	0.93	-	0.026	-	-	0.85	0.04	-	-	-	1^{st} der, SNV	Marte *et al.* (2009)
	-	-	-	-	-	-	-	0.85	-	0.04	-	-	1^{st} der, SNV	Marte *et al.* (2009)
Extract (%)	-	-	-	-	-	-	-	0.85	-	4.70	-	-	-	Henry (1985a)

SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate and de-trending; 1^{st} der=first derivative

Table 2.11 Results obtained for NIR prediction of extract, FAN and TSN from wort

Parameter	Calibration set					Validation set						Wavelengths	Pre-treatment	Reference
	Range	SD	R^2	SECV	RMSECV	Range	SD	r^2	SEP	RMSEP	RPD			
Extract (%)	58.8-84.1	-	0.94	-	-	67.1-79.9	-	0.88	0.9	-	-	4 wavelengths	-	Ratcliffe & Panozzo (1999)
FAN (mg/L)	90-320	-	0.86	-	-	105-250	-	0.73	15	-	-	4 wavelengths	-	Ratcliffe & Panozzo (1999)
TSN (%)	2.27-7.95	-	0.89	-	-	3.28-6.30	-	0.80	0.30	-	-	4 wavelengths	-	Ratcliffe & Panozzo (1999)

FAN=free amino nitrogen; TSN=total soluble nitrogen; SD=standard deviation; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of performance; RMSEP=root mean square error of performance; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation

10. Genotype-by-environment interactions

Seasonal variation in barley quality has been identified as a major problem in the brewing industry and can predominantly be attributed to differences in genotype and environmental conditions (Savin & Molina-Cano, 2002). South African malting barley is cultivated in two restricted regions (dry land and irrigation) (Kotze, 2009b) and the effect of different production factors such as cultivar choice, planting date, nitrogen fertilisation and irrigation are reflected in the yield as well as the quality of the crop (Kotze, 2009a).

Nitrogen content and kernel plumpness are both genetically and environmentally controlled (Fox *et al.*, 2006). Low kernel plumpness results from unfavourable conditions during grain filling and may also be a function of a cultivar's propensity for low plumpness (Kotze, 2009b). The β-glucan and starch contents of a barley grain also vary with cultivar and environment (Duffus & Cochrane, 1993). The selection of cultivars with superior quality is thus not simple, due to differential genotypic expression across environments and the genotype-by-environment (GxE) interaction has important implications in breeding programmes, including specific adaptation and choice of location for selection as well as resource allocation in advanced line testing across sites/years (Voltas *et al.*, 2002).

GxE interaction is reflected in the various responses of genotypes to environmental conditions. The effects of the GxE interaction may depend on the genetic background of the genotypes and on the degree of differentiation of environmental conditions. Malting quality decreases in hot dry environments and in this case GxE effects may be observed when genotypes differ in their susceptibility to such environmental conditions (Kaczmarek *et al.*, 1999). The GxE interaction weakens the association between genotype and phenotype and may reduce genetic progress in breeding programmes. Averaging across different environments ignores that genotype performance may be a function of environment and is only an adequate indicator of genotypic performance if GxE interaction is not present. For trials in which genotypes and locations are repeated across years, GxE interactions can be assessed via the use of ANOVA or visual tools such as PCA biplots. Such a test must be based on a series of trials carried out over adequate locations and years to represent the intended area for the breeding programme (Voltas *et al.*, 2002). PCA biplots which show both genotypes and environments simultaneously (Gabriel, 1971) allow for the display of those dimensions which account for the maximum amount of variation in which the GxE interaction is presented as well as possible on the same graph. This is useful for investigating the response of lines over different environments (Kempton, 1984). In these biplots, genotype markers are represented by points and environment markers by vectors (Kempton, 1984; Kroonenberg, 1995). The relationships or interactions of two genotypes with the same environment can be assessed by comparing the lengths of their projections onto that environment. Furthermore, the relationship or interaction between a genotype vector and an environment vector is positive if their angle is acute (less than 90°) and negative in the case of an obtuse angle (90-180°). When the projection of a genotype marker onto the environment vector coincides with the origin (0), the

interaction is negligible. A positive value indicates that the genotype has a high score in the environment relative to the average score in that environment, and a negative value indicates that the genotype has a relatively low score in the environment (**Fig. 2.3**) (Kroonenberg, 1995; Voltas *et al.*, 2002). If two genotype points have a small angle, they have similar response patterns over environments and if two environment vectors have a small angle they are strongly associated (Kroonenberg, 1995).

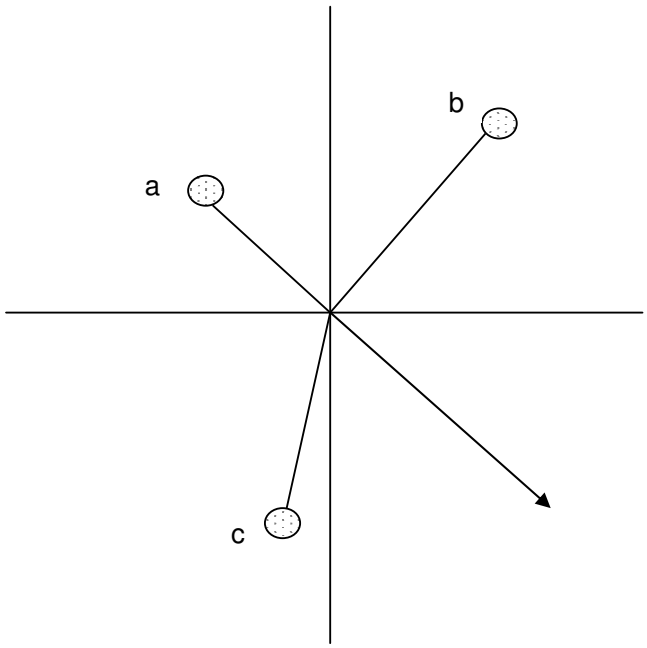

Figure 2.3 Biplot representation of genotype markers (○) and an environment vector (↗) indicating a) an obtuse angle (91°-180°), b) a right angle (90°) and c) an acute angle (0°- 89°).

A GxE study carried out in Spain combined the results of five different lines and six locations over two growing seasons and included the malt properties extract, TN, KI, wort viscosity and AAL. Results showed that genotype had a significant ($P \leq 0.05$) effect on all properties except TN, while the effect of location was significant ($P \leq 0.05$) for all characteristics except for AAL. Year effects were significant ($P \leq 0.05$) for TN, KI and AAL. TN was also affected by a location x year interaction. Malt extract and viscosity were shown to be governed mainly by the genotype while malt protein depended on location and KI on climatic conditions of the year. It was concluded that extract and wort viscosity were mainly influenced by genotype while TN depended mainly on the location. Although no significant GxE interactions were observed, viscosity and TN showed a significant positive correlation ($P \leq 0.01$), while viscosity and extract showed a significant negative correlation ($P \leq 0.05$). Extract and KI showed a significant positive correlation ($P \leq 0.01$) while TN showed a significant negative correlation ($P \leq 0.01$) for both extract and KI. A significant positive correlation ($P \leq 0.05$) existed between KI and AAL (Molina-Cano *et al.*, 1997).

Correlations between agronomic and malting quality traits were calculated from a population of 102 malting barley lines (Rutger *et al*., 1967). Plumpness and malt extract were positively correlated ($P \leq 0.01$) while a significant negative correlation existed between plumpness and DP ($P \leq 0.01$) and plumpness and TN ($P \leq 0.01$). Malt extract was negatively correlated with DP ($P \leq 0.01$) while TN was positively correlated with DP ($P \leq 0.01$) (Rutger *et al*., 1967).

Five barley cultivars were evaluated for correlations between barley nitrogen percentage, extract and DP and the crosses were grown over two years. Nitrogen and DP showed a consistent positive correlation ($P \leq 0.01$) with each other, but significant negative correlations with extract ($P \leq 0.01$). The authors stated that the negative association between extract and barley nitrogen content is to be expected as an increase in one necessitates a reduction in the other (Foster *et al*., 1967).

Den Hartog and Lambert (1953) determined the relationship between nitrogen, DP and extract in 10 early generation crosses of a barley breeding programme and found these malt properties to be closely related. Nitrogen content was positively correlated with DP and negatively correlated with extract, with the correlation between DP and extract being negative. DP and extract were believed to be related because of their association with protein. Significant differences ($P \leq 0.01$) existed between crosses for each of the three properties (Den Hartog & Lambert, 1953).

A study carried out in South East Australia calculated GxE interactions for grain nitrogen, extract and DP. The study included seven cultivars grown at the same location over two seasons, with a large temperature and rainfall difference between the two seasons. Difference in season proved to have a significant effect on all quality properties (($P \leq 0.05$) for protein as well as extract, and for DP ($P \leq 0.01$)) with season x cultivar being highly significant ($P \leq 0.05$) for all three properties. Cultivar had a significant effect on extract ($P \leq 0.01$) and on DP ($P \leq 0.05$). Barley nitrogen content and extract were found to be negatively correlated for both the environmental and genotypic correlation, while the genotypic correlation between extract and DP was positive (Eagles *et al*., 1995).

The influence of genotype and location on DP was investigated by Arends *et al.* (1995), and both proved to have an influence on DP. The study included 11 cultivars grown over six locations and grain nitrogen content was found to be positively correlated with DP ($P \leq 0.05$). High grain nitrogen content and DP also showed a significant negative correlation with low extract values ($P \leq 0.05$) (Arends *et al*., 1995).

The malt properties nitrogen content, KI and extract were included in a study in Poland, where 30 lines were produced over two years in three locations. A great difference existed between the soil type and temperature of the locations. The highest GxE interaction was found for KI and extract, while 20 of the lines showed no interaction between the environment and nitrogen content. The GxE interaction as well as the interaction of environment only was significant ($P \leq 0.01$) for all three parameters (Kaczmarek *et al*., 1999).

A study to assess the effect of cultivar and environment on barley quality revealed that cultivar was the most significant factor affecting β-glucan content; environmental effects were found to be less important. Ten barley cultivars grown in two seasons were evaluated and environment, cultivar and the cultivar x environment interactions influenced β-glucan and nitrogen content significantly. The environmental factor was of special importance for barley nitrogen content (Oscarsson *et al.*, 1998).

11. Conclusion

The selection of high quality malting cultivars and the effective quality determination thereof is of paramount importance to insure the highest quality end product is obtained. NIR spectroscopy is a technique with great potential for quality evaluation in breeding programmes consisting of thousands of lines and cultivars that require fast and effective evaluation. Although NIR prediction from whole grain barley cannot account for enzyme action during malting (possibly due to the complex nature of the constituents and the fact that unmalted barley contains proteins and starches which are not yet modified by the action of enzymes during malting), the technique shows potential to be used as a screening method in earlier generations. This would allow for the elimination of poor malting cultivars in early stages of the breeding programme. The use of NIR spectroscopy holds several advantages, especially when considered for quality assessment where limited samples sizes rule out the possibility of other predictive tests such as micro-malting.

It is important to ensure a wide range of samples, including all variability to be expected in future predictions, are obtained for calibration development. A wide range of samples including all properties to be expected in future samples, is possible if samples are taken from different years, locations and cultivars as different growing seasons will provide unique conditions. This will also allow for the determination of GxE interactions and correlations between different malting properties, which in turn can provide information on consistency in quality over seasons and localities, and allow breeders to select suitable lines to make the transition to commercial cultivars.

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Chapter 3

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Abstract

Near infrared (NIR) spectroscopy calibration models were developed for the prediction of barley quality properties (plumpness and moisture content) and malt quality properties (extract, total nitrogen (TN), total soluble nitrogen (TSN), Kolbach Index (KI), free amino nitrogen (FAN), diastatic power (DP), wort viscosity, apparent attenuation limit (AAL) and wort β-glucan content) from whole grain and ground South African barley using three different spectrometers and two data analysis software packages. Whole grain reflectance spectra were obtained with Büchi NIRFlex N-500 and Bruker MPA spectrometers using The Unscrambler and OPUS software for data analysis, respectively. Reflectance spectra of whole grain and flour samples were also recorded with a Büchi NIRLab N-200 spectrometer and The Unscrambler was used for data analysis. Using principal component analysis, it was possible to distinguish between irrigation and dry land samples, as well as between samples cultivated at specific localities. Whole grain calibration models appropriate for screening or rough screening were developed for the properties plumpness, extract, TN, TSN, KI, FAN and wort β-glucan content from the irrigation samples. For the dry land samples, models for the properties moisture content, extract, TN, TSN, FAN and DP were developed. For flour samples, models acceptable for screening or at least rough screening, were developed for the irrigation sample properties moisture content, plumpness, extract, TN, TSN, FAN, DP and wort β-glucan content. Dry land flour models for moisture content, TN, TSN, FAN and wort viscosity were acceptable for screening or rough screening. AAL was the only parameter that could not be predicted with accuracy appropriate for at least rough screening purposes for all instruments or sample types. The use of uncertainty testing for wavelength selection only showed improvement in some cases, i.e. whole grain models improved to a level acceptable for screening, included moisture content, FAN and DP for dry land samples as well as extract, TN and KI for irrigation samples. Wort viscosity and β-glucan content were the only properties for which variable selection improved a flour model. The use of flour samples in calibration development showed an improvement over whole grain samples for moisture content, TN, TSN, KI, FAN, wort viscosity and β-glucan content for dry land and irrigation samples. In the case of DP, irrigation samples showed more accurate prediction for flour, but dry land samples showed a more accurate prediction for whole grain while plumpness, extract and AAL delivered similar results for whole grain and flour models. Dry land samples delivered better results in the case of moisture content, TSN, FAN, DP and wort viscosity. AAL delivered similarly poor results for both dry land and irrigation samples. Better results were obtained with irrigation samples for wort β-glucan content, plumpness, extract, TN and KI. The addition of a second harvest season to calibration development did not show much improvement in calibration results based on the 2008 season alone.

Introduction

Malting barley is the second most important small grain in South Africa and is grown under irrigation in the Northern Cape or under dry land conditions in the Southern Cape (Kotze, 2009). In malting barley breeding programmes, a great number of lines (*ca.* 3000) must be tested for their malting quality in a short time, and results should be reliable and consistent over years and locations (Osborne, 2006). The assessment of many barley and malt quality characteristics is required, and often these tests require larger samples of barley than are available in earlier generations of the breeding programme. South African breeders currently use near infrared (NIR) spectroscopy in transmittance mode (800-1100 nm) for limited quality evaluation, i.e. prediction of nitrogen and moisture contents of whole grain barley. In addition, micro-malting techniques are used to evaluate the malting potential of selected lines. Micro-malting involves the malting of barley on a small scale to provide an indication of malting quality potential. This allows breeders to determine which lines possess adequate properties to progress to the next stage in the breeding programme. However, micro-malting is a time consuming and laborious process. It is also destructive, requires large amounts of grain (200 g) and can only be used at later stages in the breeding programme (F. Smit, South African Barley Breeding Institute (SABBI), Caledon, South Africa, Personal Communication, 2009).

NIR spectroscopy is based on absorption of electromagnetic radiation in the NIR wavelength range (750-2500 nm) (Butler, 1983; Osborne, 2000). NIR spectroscopy in reflectance mode uses the 1100 to 2500 nm wavelength range and can be applied to whole and ground grain. It can be implemented for quality testing as early as the F5 generation as 100 g of seed would be available. It is also a fast, reliable and non-destructive technique that does not require large sample sizes (Osborne, 1981). This technique could allow breeders to predict the malting quality of unmalted breeding lines with suitable quality characteristics.

Exploratory analyses such as principal component analysis (PCA) can be applied to spectral data to examine relationships between samples and spectra (Cowe & McNicol, 1985). Quantitative chemometric techniques such as partial least square (PLS) regression are used in calibration development and establish a linear relationship between NIR spectra and reference data (Pasquini, 2003). An NIR calibration model is developed by selecting a set of calibration samples with known chemical and/or physical property values covering the range expected in future unknown samples. The accuracy of this mathematical relationship may be tested using the NIR spectra of independent samples (validation/test set) (Bokobza, 1998; Cen & He, 2007). Uncertainty testing is based on significance testing of model parameters to eliminate useless or unreliable variables in the NIR spectra and simplify the final model (Westad & Martens, 2000; Esbensen, 2002). If the predictability of a simpler model with fewer variables is as good as, or better, than that of the full model, the simpler model is preferred (Esbensen, 2002).

The NIR technique is advantageous as it offers the potential to conduct rapid tests on small samples of ground grain or non-destructively on whole grain (Woodcock *et al.*, 2008); its

reproducibility is equal to and sometimes better than reference measurements (Williams, 2007); it allows for the simultaneous measurement of multiple quality properties (Sissons *et al.*, 2006) as it is not necessary to repeat scans for each constituent (McClure & Tsuchikawa, 2007); it is fast (one minute per sample), non-invasive and requires no sample preparation (Pasquini, 2003). Major disadvantages of NIR include its dependence on often complicated and expensive reference methods (Osborne, 2000), and calibrations that are dependent on sophisticated chemometric techniques; both of which require the expertise of highly skilled personnel (McClure & Tsuchikawa, 2007). The most apparent disadvantage is that separate calibrations are needed for each property (Williams, 2007).

NIR reflectance spectroscopy based calibration models for the prediction of malt quality from whole and ground malt, as well as wort, have been calculated (Henry, 1985; Ratcliffe & Panozzo, 1999; Black & Panozzo, 2001; Marte *et al.*, 2009). Prediction of barley and malt quality characteristics with NIR reflectance spectroscopy have included whole grain unmalted barley analyses for plumpness (Edney *et al.*, 1994), moisture (Downey, 1985; Halsey, 1987; Li *et al.*, 1995; Sohn *et al.*, 2008), β-glucan (Black & Panozzo, 2001; Sohn *et al.*, 2008), nitrogen contents (Halsey, 1987; Edney *et al.*, 1994; Li *et al.*, 1995; Sohn *et al.*, 2008), diastatic power (DP) (Li *et al.*, 1995; Black & Panozzo, 2001), free amino nitrogen (FAN), total soluble nitrogen (TSN) (Black & Panozzo, 2001), as well as extract (Halsey, 1987; Li *et al.*, 1995; Black & Panozzo, 2001) and wort viscosity (Li *et al.*, 1995). Studies on ground unmalted barley have included the prediction of nitrogen (Gill *et al.*, 1979; Henry, 1985), moisture (Downey, 1985; Henry, 1985b) and β-glucan contents (Allison *et al.*, 1978; Henry, 1985; Szczodrak *et al.*, 1992), as well as malt extract (Morgan & Gothard, 1979; McGuire, 1982; Henry, 1985). No South African studies on this topic have been reported to date.

The objective of this study was to develop NIR calibration models for the prediction of barley, as well as malt, quality properties from unmalted whole and ground barley, obtained from a South African breeding programme.

Materials and methods

Samples and sample preparation

Barley samples (n = 2082; 39 cultivars from 16 localities) were obtained from the SABBI 2008 breeding trials. Samples tested were from trials carrying breeding material from years 6 to 13 of an 18 year breeding programme and were grown either under irrigation (n = 732) or under dry land (n = 1350) conditions. For the 2009 season, barley samples (n = 535) from 25 cultivars were obtained from 13 localities and included irrigation (n = 178) and dry land samples (n = 357). Trials were designed according to the nearest neighbour with three repetitions.

Reference data

Reference data for plumpness was obtained by measuring barley above a 2.5 mm sieve (supplied by SABBI). The number of samples tested is shown in **Table 3.1**. Whole grain samples were milled on a UDY Cyclone Mill (UDY Corporation, Colorado, USA) fitted with a 1 mm sieve.

Barley moisture content was determined according to the European Brewery Convention (EBC) method 4.2 (European Brewery Convention, 1998). Moisture dishes were dried at 106˚C for 30 minutes and subsequently allowed to cool in a dessicator for 40 min. The mass of each moisture dish was determined to the nearest 0.001 g (Precisa balance, model 205SCS, Milton Keynes, United Kingdom) and recorded (W_1), after which a sample of 5 ± 0.0001 g of ground barley was weighed into the moisture dish (W_2). All moisture dishes were placed uncovered in a vacuum oven (Heraeus Model RVT 360, Henau, Germany) for three hours at 106˚C. The dishes were removed, covered and allowed to cool in a dessicator for 40 min. The mass of the dish and flour was determined (W_3) and the moisture content (%) calculated with the equation: ($W_2 – W_3$ / $W_2 – W_1$) x 100.

Replicates from field trials were bulked and the samples (**Table 3.1**) were malted on a small scale in Seeger, Joe White or Phoenix micro-malting machines. The steep cycle was carried out with 9 hrs steeping at 15˚C, 14 hrs air rest at 17˚C, 14 hrs steeping at 15˚C and 6 hrs air rest at 17˚C followed by two germinations; 24 hrs at 19˚C and 72 hrs at 17˚C. The kilning stage was 14 hrs at 65˚C followed by 4 hrs at 80˚C. Malt was cooled down to 30˚C after which extract, total nitrogen (TN), TSN, Kolbach Index (KI), FAN, DP, wort viscosity, apparent attenuation limit (AAL) and wort β-glucan content were determined in accordance with the methods mentioned in Chapter 2.6 (H. van Wyk, South African Breweries Maltings (SABM), Caledon, South Africa, Personal Communication, 2009).

Table 3.1 Number of samples from 2008 and 2009 harvest seasons for which reference data was obtained

Property		**2008**	**2009**
Plumpness	Dry land	1092	216
	Irrigation	720	120
Moisture	Dry land	161	144
	Irrigation	106	120
Malt properties	Dry land	312	224
	Irrigation	216	125

NIR analysis (Spectral data collection)

For the 2008 season, three spectrometers were evaluated. Spectra of 2049 whole grain barley samples (1320 dry land samples; 729 irrigation samples) were recorded with Bruker MPA (Bruker South Africa (Pty) Ltd, Johannesburg, South Africa) and Büchi NIRFlex N-500 (Büchi Labortechnik AG, Flawil, Switzerland) spectrometers. Spectra were recorded in reflectance mode from 1000 to 2500 nm (12 500-4000 cm^{-1}) as averages of 32 scans. Samples were presented to the Büchi instrument in glass Petri dishes and to the Bruker MPA spectrometer in the instrument's solid cell. Spectra of 263 whole grain and flour samples (158 dry land samples; 105 irrigation samples) were recorded with the Büchi NIRLab N-200 spectrometer (Büchi Labortechnik AG, Flawil, Switzerland) and were used to form barley plumpness and moisture content calibrations. For prediction of malt properties (extract, TN, TSN, KI, FAN, DP, AAL, wort viscosity and β-glucan content) spectra of 238 whole grain and flour samples (139 dry land samples; 99 irrigation samples) were recorded with the Büchi NIRLab N-200. All samples were presented to the instrument in glass Petri dishes and spectra were recorded in reflectance mode from 1000 to 2500 nm (12 500-4000 cm^{-1}).

Only the Büchi NIRLab N-200 spectrometer was used for recording spectra of samples from the 2009 season. All samples were presented to the instrument in glass Petri dishes and spectra were recorded in reflectance mode from 1000 to 2500 nm (12 500-4000 cm^{-1}). Spectra of 264 whole grain and flour samples (144 dry land samples; 120 irrigation samples) were collected and used for moisture content predictions, while spectra of 336 whole grain and flour samples (216 dry land samples; 120 irrigation samples) were collected for prediction of plumpness as well as malt quality properties.

NIR analysis (Data analysis)

PCA was applied to the spectral data of the dry land (n = 675) and irrigation (n = 336) samples obtained with the Bruker MPA instrument, using the OPUS (Version 6.5, Bruker MPA Optics GmbH, Germany) data analysis software. The effect of growing conditions (dry land and irrigation) as well as the effect of locality was studied. No pretreatment was applied to the spectral data.

PLS models were developed using the Büchi NIRFlex N-500 data with The Unscrambler (Version 9.2, CAMO, Oslo, Norway) data analysis software and from the Bruker MPA data with OPUS data analysis software. This was carried out for the barley quality properties plumpness and moisture content, as well as the malt quality properties, i.e. extract, TN, TSN, KI, FAN, DP, AAL, wort viscosity and β-glucan content. An independent validation set (**Table 3.2**) was chosen for plumpness and moisture content by selecting every third value from a list of ascending values for the respective properties. Samples used in calibration development for the malt properties were split into a fixed calibration and test set (**Table 3.2**) with the Kennard and Stone algorithm (Daszykowski *et al.*, 2002), which allowed for the selection of a representative subset of samples for the training set. PLS models were developed from the Büchi NIRLab N-200 spectra with The Unscrambler data analysis software. An independent validation set (**Table 3.3**) was chosen for

plumpness, moisture content and all malt properties by selecting every third value from a list indicating ascending values for the respective property. All calibration models developed were evaluated by means of full-cross validation; whereafter the models with the best results were validated with a test set. A number of pretreatment techniques (**Table 3.4**) were applied and the Büchi NIRFlex N-500 and NIRLab N-200 models were validated using test sets as well as uncertainty testing with segmented cross-validation (3 samples per segment) (Martens & Martens, 2000) in The Unscrambler (uncertainty testing refers to the process of spectral variable selection). Pre-treatments delivering the best results for each parameter, were applied before segmented cross-validation with uncertainty testing and the variables that proved to be most significant from uncertainty testing were evaluated with test set validation. The Bruker MPA data was evaluated by test set validation only. The two software packages offered different pretreatment options (**Table 3.4**).

PLS models were also developed with The Unscrambler data analysis software by combining sample spectra (**Table 3.3**) from two harvest seasons (2008 and 2009) scanned on the Büchi NIRLab N-200. This was performed on moisture content, plumpness and the malt quality properties i.e. extract, TN, TSN, KI, FAN, DP, AAL, wort viscosity and β-glucan content. All calibration models developed were evaluated by means of full-cross validation, where after an independent validation set (**Table 3.3**) was chosen only for moisture content, TN and TSN by selecting every third value from a list of ascending values for the respective properties (these were the only properties for which R^2 for cross validation was higher than 0.50).

The accuracy of each calibration model was determined from the standard error of prediction (SEP), the coefficient of determination (r^2) and the ratio of the SEP to the standard deviation of the validation set (RPD), where the aim is to obtain the lowest SEP with the highest r^2 and RPD values (Williams, 2001).

Table 3.2 Number of samples used for calibration and validation sets of whole grain samples scanned with the Büchi NIRFlex N-500 and Bruker MPA instruments

		Total	Calibration set	Validation set
Plumpness	Dry land samples	1092	729	363
	Irrigation samples	729	499	230
Moisture content	Dry land samples	161	109	52
	Irrigation samples	106	72	34
Malt properties	Dry land samples	312	210	102
	Irrigation samples	216	144	72

Table 3.3 Number of samples used for calibration and validation sets of whole grain and flour samples scanned with the Büchi NIRLab N-200 instrument

			Total	Calibration set	Validation set
2008 Samples	Moisture	Dry land samples	158	106	52
		Irrigation samples	99	65	34
	Plumpness	Dry land samples	158	106	52
		Irrigation samples	99	65	34
	Malt properties	Dry land samples	139	95	44
		Irrigation samples	99	68	31
2008 + 2009 Samples	Moisture	Dry land samples	301	202	99
		Irrigation samples	223	150	73
	TN	Dry land samples	355	239	116
		Irrigation samples	213	145	68
	TSN	Dry land samples	355	239	116
		Irrigation samples	213	147	66

TN=total nitrogen; TSN=total soluble nitrogen

Table 3.4 Pretreatment techniques used in calibration development for the three respective instruments

Büchi NIRFlex N-500 and NIRLab N-200 (The Unscrambler)	**Bruker MPA (OPUS)**
No spectral pretreatment	No spectral pretreatment
Mean normalization	Min max normalization
Standard Normal Variate (SNV)	Vector normalization (SNV)
1st derivative[a], 9 points	1st derivative, 9 points
2nd derivative[b], 17 points	2nd derivative, 17 points
1st derivative, 9 points and SNV	1st derivative, 9 points and SNV
2nd derivative, 17 points and SNV	1st derivative, 9 points and MSC

[a] 1st derivative Savitzky-Golay

[b] 2nd derivative Savitzky-Golay

Results

Reference data

A summary of the reference data for the dry land and irrigation whole grain samples, scanned on both the Büchi NIRFlex N-500 and Bruker MPA instruments, can be seen in **Tables 3.5** and **3.6**, respectively. Histograms of the reference value distributions for the dry land and irrigation samples scanned on these two instruments are shown in **Figs. 3.1** and **3.2**. Summaries of the reference data for the dry land and irrigation samples scanned on the Büchi NIRLab N-200 are shown in **Tables 3.7** and **3.8** (both whole grain and flour samples were scanned), while histograms of the reference value distributions for these dry land and irrigation samples are shown in **Figs. 3.3** and

3.4. Moisture content, TN and TSN are summarised for the sample set combining 2008 and 2009 harvests in **Tables 3.9** and **3.10**, while histograms of these reference value distributions are shown in **Figs. 3.5** and **3.6**.

Table 3.5 Summary of the reference data for dry land sample properties (scanned using Büchi NIRFlex N-500 and Bruker MPA)

Properties	Total sample set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Plumpness (%)	1092	78.0 - 99.1	93.38	4.12	729	78.0 - 99.1	93.48	3.80	363	78.7 - 98.9	93.51	3.71
Moisture (%)	161	8.20 - 12.59	10.33	0.96	109	8.20 - 12.59	10.33	0.97	52	8.49 - 12.53	10.33	0.94
Extract (%)	312	78.4 - 83.4	80.64	1.08	210	78.4 - 83.4	80.68	1.13	102	79.2 - 82.4	80.55	0.97
TN (%)	312	1 - 2.05	1.58	0.24	210	1 - 2.05	1.58	0.26	102	1.08 - 1.98	1.59	0.22
TSN (%)	312	0.45 - 0.95	0.68	0.12	210	0.45 - 0.92	0.68	0.12	102	0.49 - 0.95	0.67	0.12
KI	312	34 – 52	42.96	3.82	210	34 - 50	43.19	3.36	102	34 - 52	42.49	4.61
FAN (mg/L)	312	107 - 286	187.92	40.28	210	107 - 284	191.30	37.87	102	117 - 286	180.98	44.22
DP (W.K.)	312	170 - 635	392.60	112.57	210	173 - 635	390.51	112.79	102	170 - 635	396.88	112.56
Viscosity (cP)	312	1.4 - 1.64	1.47	0.03	210	1.4 - 1.64	1.47	0.03	102	1.42 – 1.64	1.47	0.03
AAL (%)	312	78.2 - 89.8	84.65	2.09	210	78.2 - 89.8	84.80	2.17	102	78.2 – 88.7	84.35	1.91
β-glucans (mg/L)	312	49 - 342	89.95	52.73	210	49 - 342	79.07	35.91	102	55 - 342	112.34	71.69

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach Index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; SD=standard deviation

Table 3.6 Summary of the reference data for the irrigation sample properties (scanned using Büchi NIRFlex N-500 and Bruker MPA)

Properties	Total sample set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Plumpness (%)	729	63.7 - 99.6	93.71	5.09	499	63.7 – 99.6	93.63	5.37	230	71.0 – 99.4	93.87	4.49
Moisture (%)	106	6.95 – 10.81	9.15	0.69	72	6.95 – 10.81	9.13	0.70	34	8.03 – 10.64	9.17	0.66
Extract (%)	216	77.6 – 83.6	81.08	1.13	144	79.3 – 83.6	81.17	0.99	72	77.6 – 83.4	80.91	1.36
TN (%)	216	1.28 – 2.08	1.59	0.17	144	1.28 – 1.99	1.56	0.15	72	1.28 – 2.08	1.66	0.20
TSN (%)	216	0.46 – 0.96	0.66	0.11	144	0.5 – 0.96	0.64	0.10	72	0.46 – 0.87	0.69	0.12
KI	216	32 – 51	40.93	4.29	144	32 – 51	40.65	4.06	72	32 – 50	41.5	4.68
FAN (mg/L)	216	99 – 252	161.90	36.31	144	111 – 224	157.83	30.53	72	99 – 252	170.04	44.88
DP (W.K.)	216	170 – 554	357.89	88.11	144	188 – 554	366.94	75.81	72	170 – 542	339.79	106.93
Viscosity (cP)	216	1.43 – 1.6	1.47	0.029	144	1.43 – 1.54	1.47	0.021	72	1.44 – 1.6	1.48	0.04
AAL (%)	216	77 – 88	82.65	2.33	144	77 – 88	82.57	2.52	72	78 – 85.5	82.80	1.90
β-glucans (mg/L)	216	35 - 439	119.38	86.48	144	35 - 423	102.92	63.92	72	37 - 439	152.29	113.01

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach Index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; SD=standard deviation

Table 3.7 Summary of the reference data for the dry land sample properties (scanned using Büchi NIRLab N-200)

Properties	Total samples set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Moisture (%)	158	8.20 – 12.59	10.32	0.95	106	8.20 – 12.59	10.32	0.97	52	8.49 – 12.53	10.31	0.93
Plumpness (%)	158	75.6 – 98.8	93.19	4.29	106	75.6 – 98.8	93.11	4.43	52	81.1 – 98.5	93.36	4.03
Extract (%)	139	78.4 – 83.4	80.76	1.17	95	78.4 – 83.4	80.78	1.22	44	78.9 – 82.8	80.72	1.06
TN (%)	139	1 – 2.05	1.54	0.25	95	1 – 2.05	1.54	0.26	44	1.09 – 1.98	1.53	0.23
TSN (%)	139	0.45 – 0.95	0.66	0.12	95	0.45 – 0.95	0.67	0.12	44	0.49 – 0.88	0.66	0.11
KI	139	34 – 52	43.30	3.71	95	34 - 52	43.28	3.86	44	36 – 48	43.32	3.40
FAN (mg/L)	139	107 – 286	184.81	37.1	95	107 - 286	185.39	39.11	44	119 – 262	183.55	32.73
DP (W.K.)	139	170 – 635	367.73	115.59	95	170 - 635	368.90	120.39	44	173 – 595	365.23	105.77
Viscosity (cP)	139	1.4 – 1.64	1.47	0.029	95	1.4 – 1.64	1.47	0.031	44	1.42 – 1.52	1.465	0.02
AAL (%)	139	78.2 – 89.8	84.89	2.10	95	78.2 – 89.8	84.90	2.23	44	81.4 – 88.8	84.87	1.8
β-glucans (mg/L)	139	49 - 342	85.76	49.66	95	49 - 342	88.73	56.69	44	55 - 225	79.34	28.76

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach Index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; SD=standard deviation

Table 3.8 Summary of the reference data for the irrigation sample properties (scanned using Büchi NIRLab N-200)

Properties	Total sample set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Moisture (%)	99	7.89 – 10.81	9.15	0.69	65	7.89 – 10.81	9.15	0.72	34	8.03 – 10.47	9.14	0.63
Plumpness (%)	99	39.8 – 99.5	92.04	7.76	65	39.8 – 99.5	91.04	8.91	34	83 – 98.2	94.13	3.83
Extract (%)	99	77.6 – 83.6	81.10	1.15	68	77.6 – 83.6	81.09	1.23	31	78.7 – 83.2	81.12	0.98
TN (%)	99	1.28 – 2.08	1.59	0.18	68	1.28 – 2.08	1.59	0.19	31	1.33 – 1.95	1.59	0.16
TSN (%)	99	0.46 – 0.96	0.66	0.11	68	0.46 – 0.96	0.66	0.12	31	0.52 – 0.87	0.66	0.10
KI	99	32 – 51	41.09	4.01	68	32 - 51	41.12	4.25	31	36 – 48	41.03	3.51
FAN (mg/L)	99	99 – 252	161.57	36.44	68	99 - 252	162.07	38.08	31	111 – 235	160.45	33.11
DP (W.K.)	99	170 – 554	359.73	85.46	68	170 - 554	359.68	89.98	31	212 – 523	359.84	76
Viscosity (cP)	99	1.43 – 1.6	1.47	0.03	68	1.43 – 1.6	1.47	0.03	31	1.44 – 1.53	1.47	0.02
AAL (%)	99	77 – 88	82.72	2.20	68	77 - 88	82.70	2.34	31	78.3 – 85.5	82.77	1.91
β-glucans (mg/L)	99	35 – 439	119.79	89.44	68	35 - 439	123.06	96.29	31	37 - 405	112.61	73.12

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach Index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; SD=standard deviation

Table 3.9 Summary of the reference data for the dry land sample properties of two seasons (2008 & 2009) combined (spectra recorded using the Büchi NIRLab N-200)

Properties	Total samples set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Moisture (%)	301	8.20 – 12.59	10.32	0.89	202	8.20 – 12.59	10.32	0.89	99	8.57 – 12.25	10.34	0.87
TN (%)	355	1 – 2.57	1.64	0.32	239	1 – 2.57	1.64	0.33	116	1.05 – 2.49	1.65	0.31
TSN (%)	355	0.45 – 1.26	0.75	0.16	239	0.45 – 1.26	0.75	0.17	116	0.49 – 1.19	0.75	0.16

TN=total nitrogen; TSN=total soluble nitrogen; SD=standard deviation

Table 3.10 Summary of the reference data for the irrigation sample properties of two seasons (2008 & 2009) combined (spectra recorded using the Büchi NIRLab N-200)

Properties	Total samples set				Calibration set				Validation set			
	n	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
Moisture (%)	223	7.55 – 10.81	8.98	0.61	150	7.55 – 10.81	9.01	0.61	73	7.74 – 10.47	8.99	0.18
TN (%)	213	1.28 – 2.08	1.66	0.19	145	1.28 – 2.08	1.67	0.20	68	1.34 – 2.03	1.67	0.18
TSN (%)	213	0.5 – 0.98	0.76	0.13	147	0.55 – 1.08	0.86	0.12	66	0.62 – 0.86	0.86	0.11

TN=total nitrogen; TSN=total soluble nitrogen; SD=standard deviation

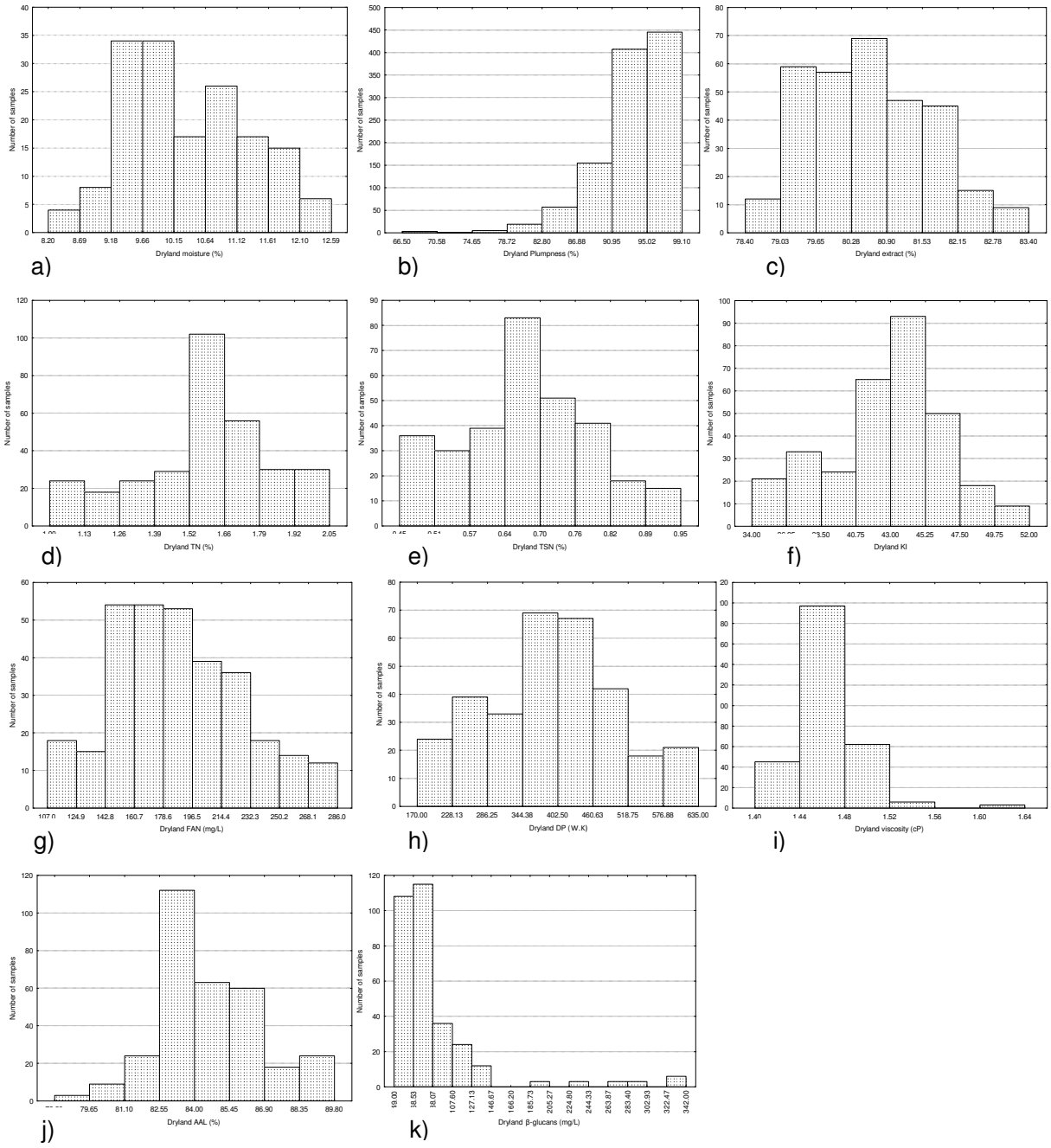


Figure 3.1 Histograms of reference value distributions for dry land samples scanned on the Büchi NIRFlex N-500 and Bruker MPA, including a) moisture, b) plumpness, c) extract, d) TN, e) TSN, f) KI, g) FAN, h) DP, i) wort viscosity, j) AAL and k) wort β-glucan values.

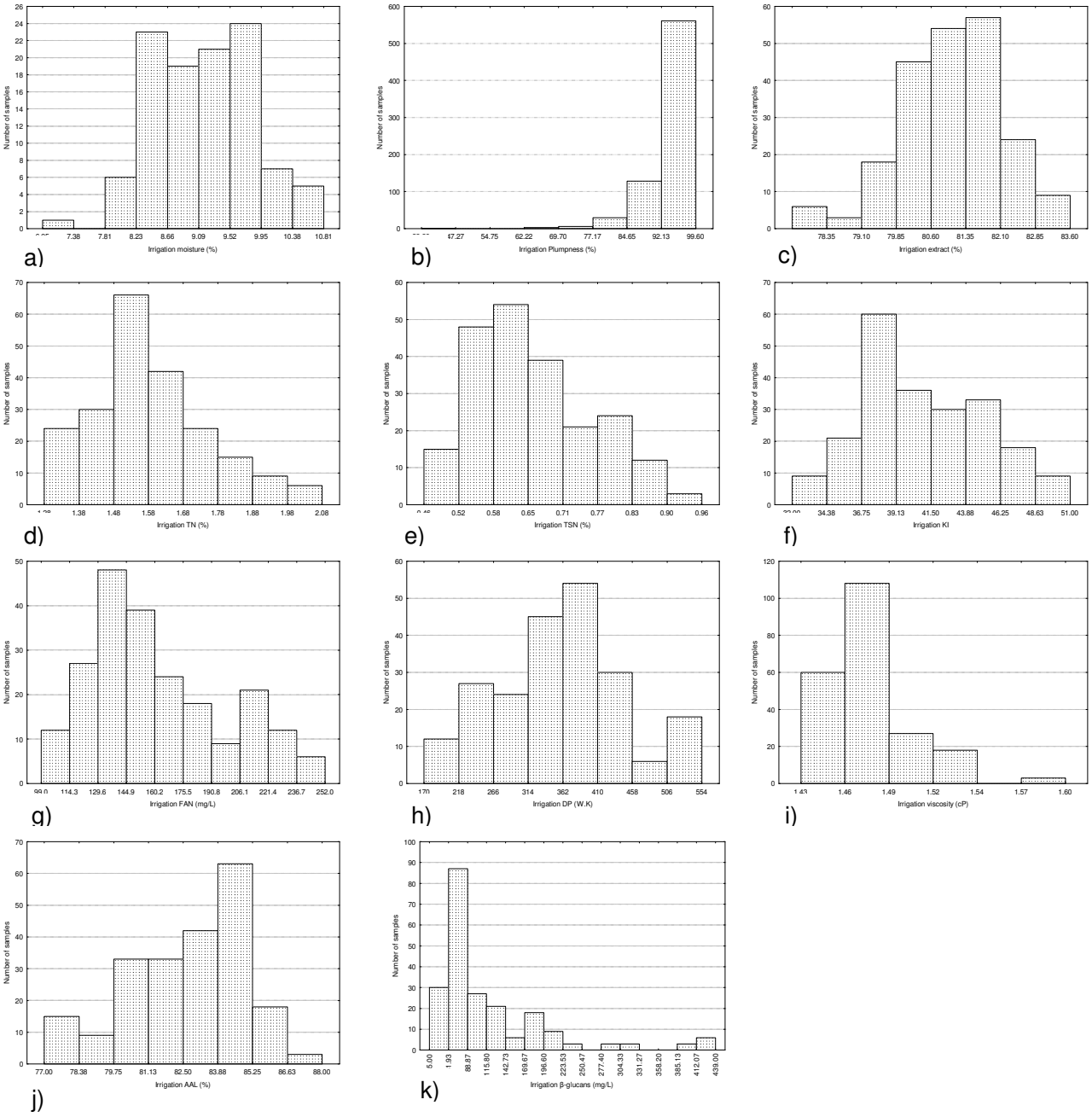


Figure 3.2 Histograms of reference value distributions for irrigation samples scanned on the Büchi NIRFlex N-500 and Bruker MPA, including a) moisture, b) plumpness, c) extract, d) TN, e) TSN, f) KI, g) FAN, h) DP, i) wort viscosity, j) AAL, k) wort β-glucan values.

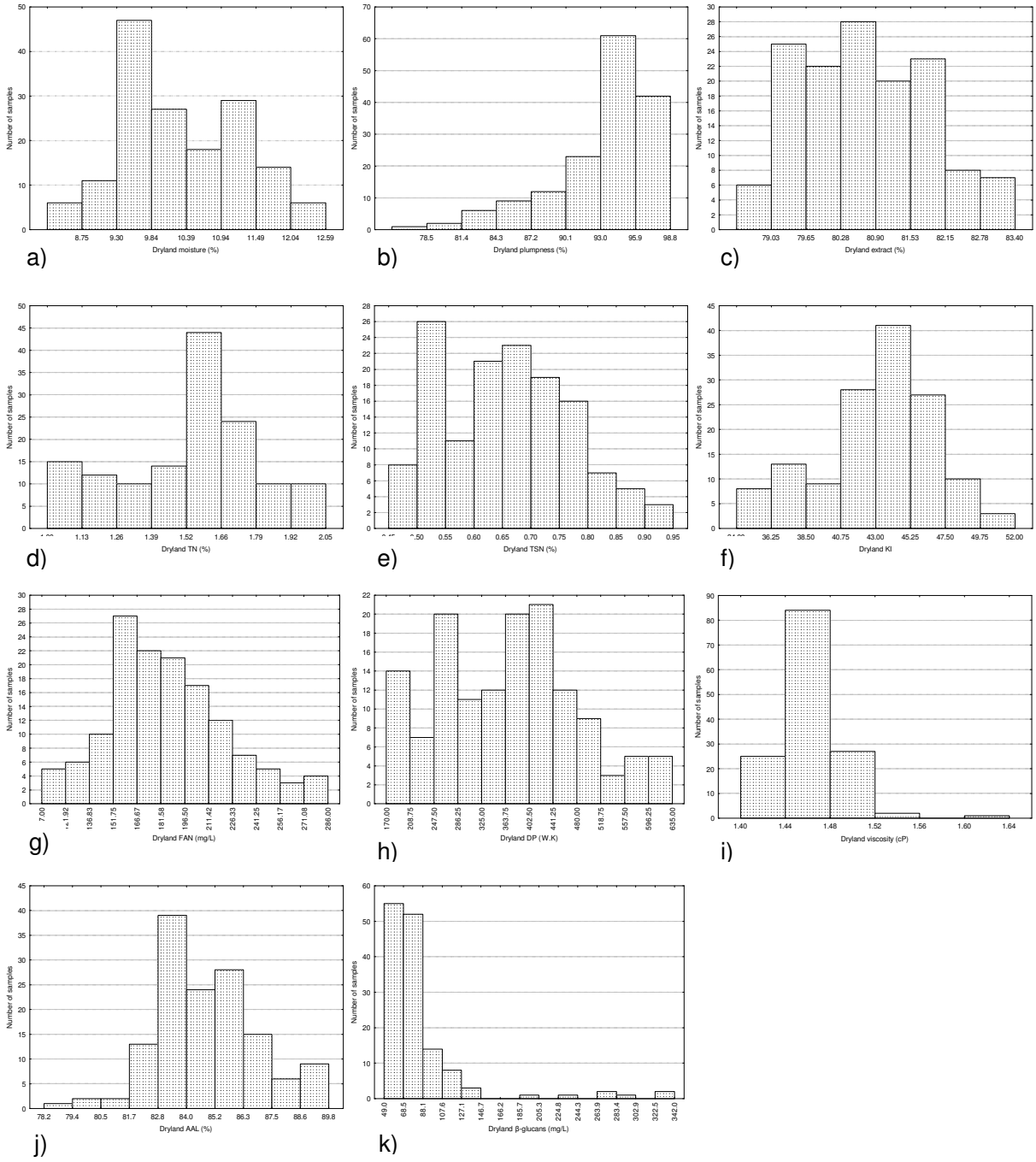


Figure 3.3 Histograms of reference value distributions for dry land samples scanned on the Büchi NIRLab N-200, including a) moisture, b) plumpness, c) extract, d) TN, e) TSN, f) KI, g) FAN, h) DP, i) wort viscosity, j) AAL and k) wort β-glucan values.

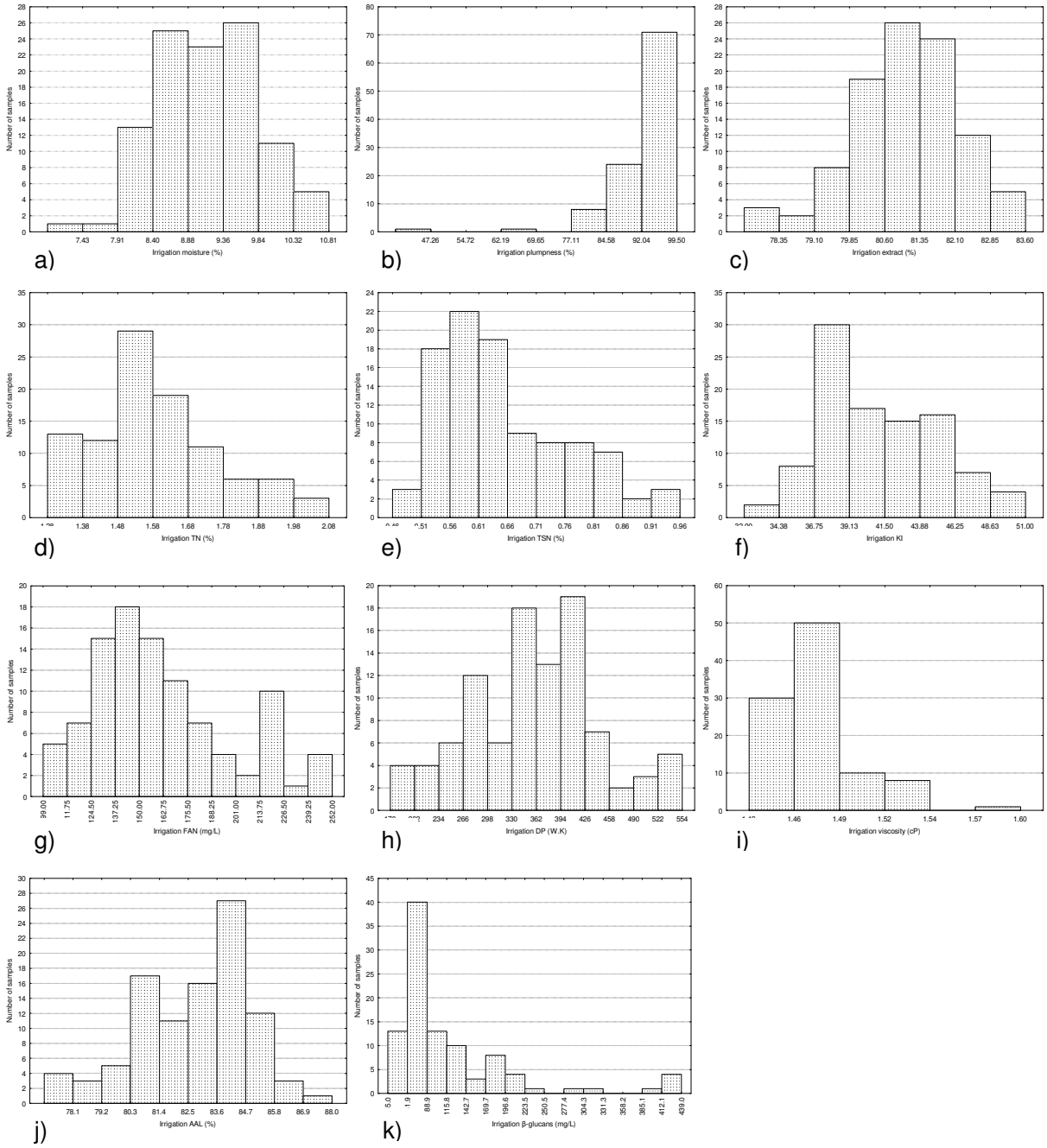
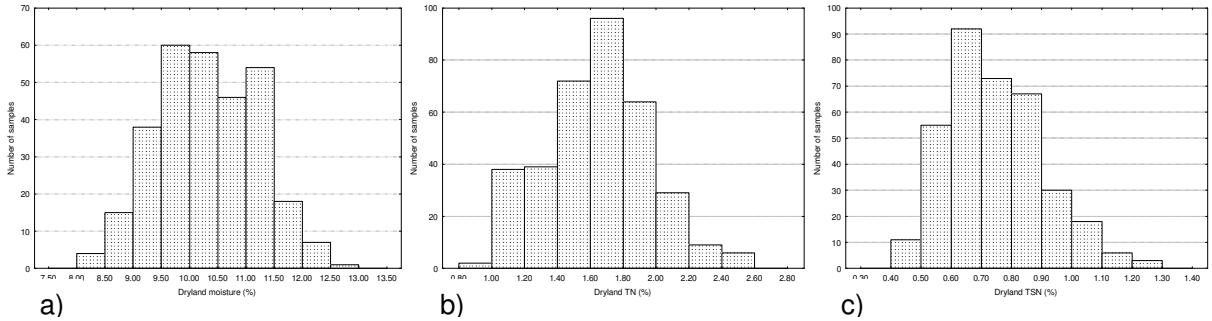
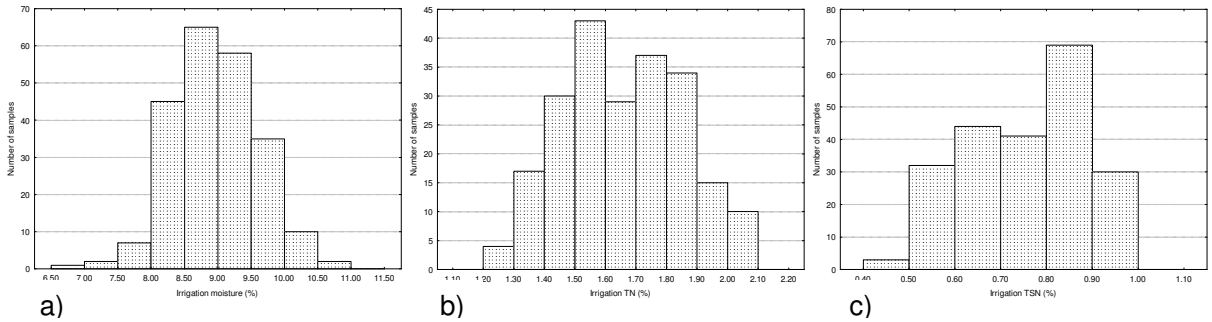


Figure 3.4 Histograms of reference value distributions for irrigation samples scanned on the Büchi NIRLab N-200, including a) moisture, b) plumpness, c) extract, d) TN, e) TSN, f) KI, g) FAN, h) DP, i) wort viscosity, j) AAL and k) wort β-glucan values.



a) b) c)

Figure 3.5 Histograms of reference value distributions for the combined 2008 and 2009 dry land samples scanned on the Büchi NIRLab N-200, including a) moisture, b) TN and c) TSN.



a) b) c)

Figure 3.6 Histograms of reference value distributions for the combined 2008 and 2009 irrigation samples scanned on the Büchi NIRLab N-200, including a) moisture, b) TN and c) TSN.

NIR analysis (spectral data collection)

Typical raw NIR spectra of whole grain and ground barley (of dry land samples) are shown in **Fig. 3.7**, where the usual effect of scattering can be observed for whole grain samples, as spectra tend to separate from each other in the higher wavelengths.

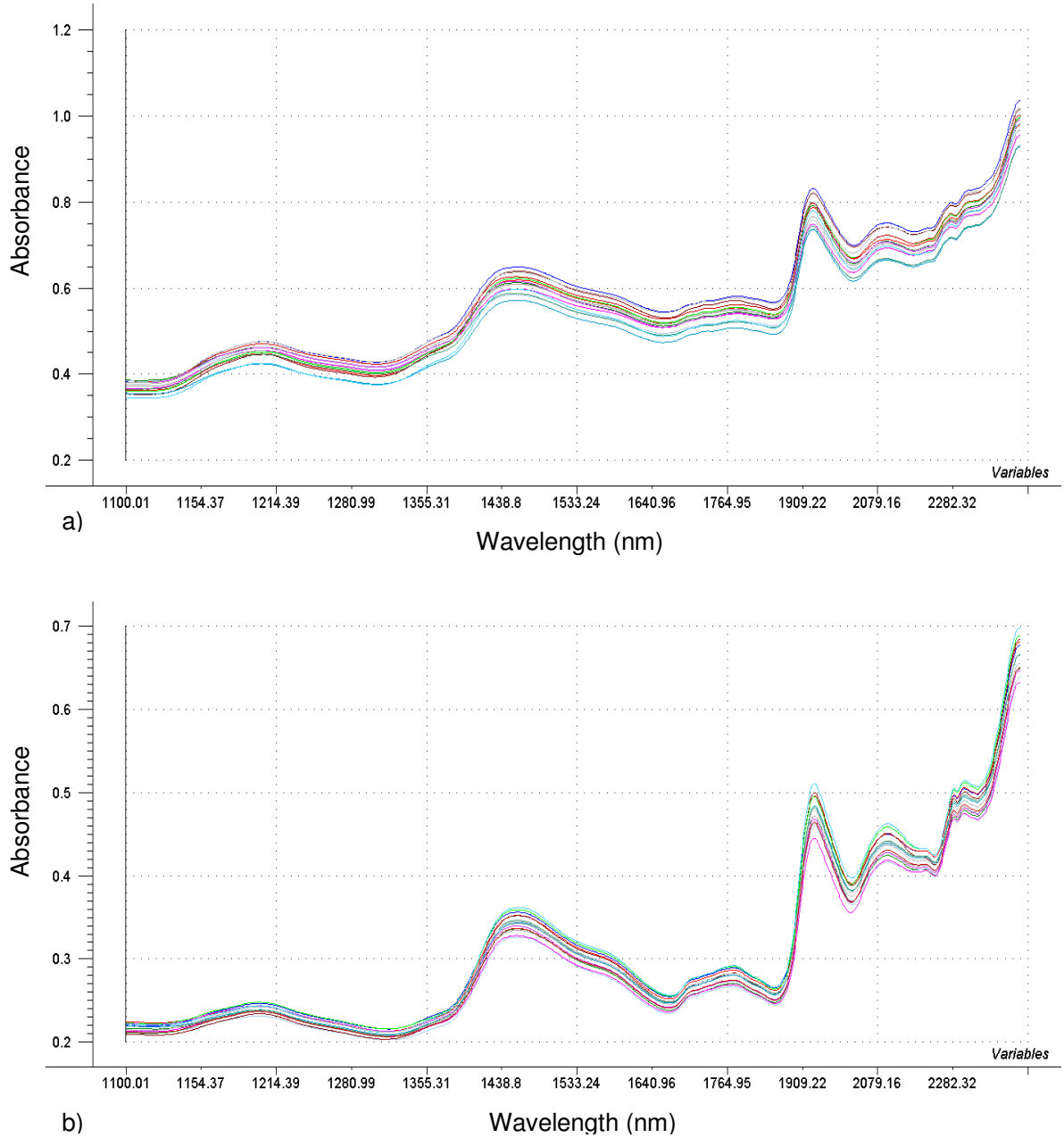


Figure 3.7 Typical raw NIR spectra (no pre-treatment) for a) whole grain barley and b) barley flour. Spectra recorded using the Büchi NIRLab N-200 instrument.

NIR analysis (data analysis)

PCA was applied to the total sample set (both dry land and irrigation samples) recorded using the Bruker MPA instrument; the PC1 vs. PC3 score plots are shown in **Fig. 3.8.** PCA was also applied to the dry land and irrigation samples sets separately; the PC1 vs. PC2 scores plots are shown in **Figs. 3.9** and **3.10**.

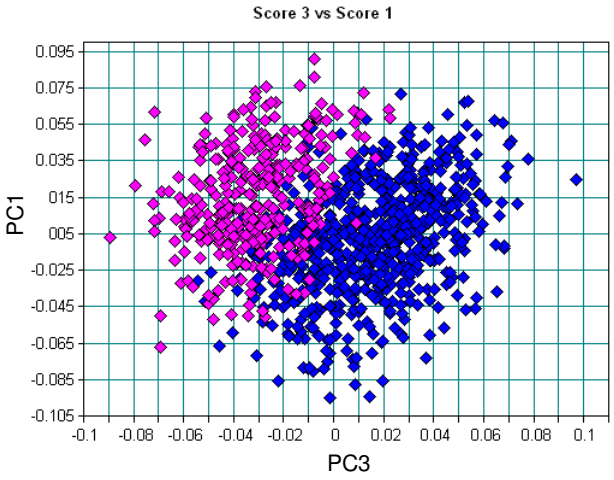


Figure 3.8 Principal component analysis score plot (PC1 vs. PC3) for dry land (blue) and irrigation (pink) samples.

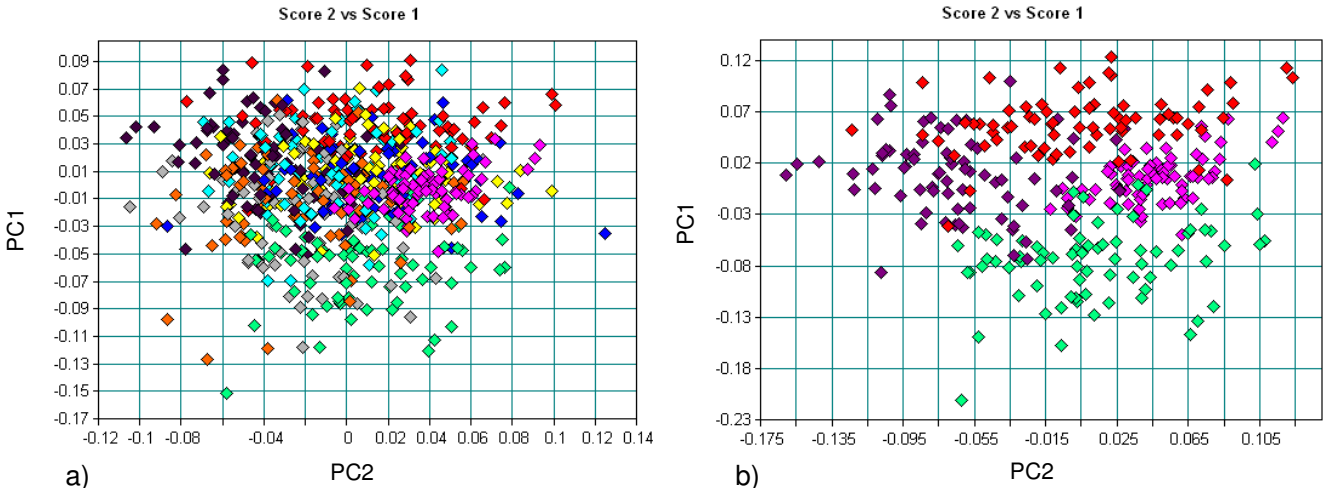


Figure 3.9 Principal component analysis score plots (PC1 vs. PC2) for samples grown under dry land conditions at (a) all the different localities and (b) at only four of the localities (Blue=Bredasdorp; Grey=Greyton; Yellow=Heidelberg; Cyan=Napier; Orange=Rietpoel; Red=Swellendam; Magenta=Klipdale; Green=Tygerhoek; Purple=Caledon).

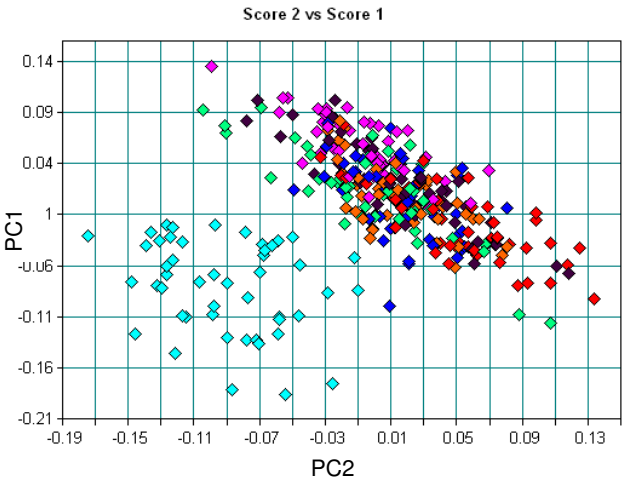


Figure 3.10 Principal component analysis score plot (PC1 vs. PC2) for samples grown at different localities under irrigation. Samples cultivated at Jan Kempdorp (cyan) could be distinguished from the remainder of the samples (Green=Taung; Orange=Hartswater; Blue=Bull Hill; Red=Rietrivier; Purple=Luckhoff; Magenta=Douglas).

Only the best models for each property, as determined by test set validation, will be discussed in more detail. All calibration results are, however, listed in **Appendix 1** (Tables 1 – 4 summarize Büchi NIRFlex N-500 data; Tables 5 and 6 summarize Bruker MPA data; Tables 7 - 13 summarize Büchi NIRLab N-200 results while the combined 2008 and 2009 cross-validation data are summarized in Tables 15 - 18).

According to Williams (2001) the coefficient of determination (r^2) should be between 0.50 and 0.64 to be acceptable for use in rough screening, while an r^2 between 0.66 and 0.81 would be acceptable for screening purposes. For a calibration to be usable in most applications, the r^2 should be above 0.83. (**Tables 2.3** and **2.4** (Chapter 2) were used for evaluation purposes). **Table 3.11** to **Table 3.13** summarize the calibration and validation results for the best test set validation models from the Büchi NIRFlex N-500 data while **Table 3.14** summarizes the Bruker MPA data. **Tables 3.15** to **3.19** summarize the calibration and validation results for the best test set validation models from the Büchi NIRLab N-200 data. The results obtained from the combination of 2008 and 2009 harvest seasons are shown in **Tables 3.20** to **3.24**.

Table 3.11 Summary of calibration and validation results for the best test set validation models from Büchi NIRFlex N-500 data

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture % (dry land)	None	0.66	0.48	0.69	0.48	-0.08	1.36	8
Moisture % (irrigation)	1st der	0.55	0.29	0.60	0.19	-0.0538	1.10	4
Plumpness % (dry land)	Mean norm	2.63	0.52	3.03	0.37	0.13	1.25	12
Plumpness % (irrigation)	Mean norm	3.70	0.03	3.47	0.07	-0.0960	1.29	2
Extract % (dry land)	2nd der & SNV	0.75	0.56	0.77	0.39	0.1930	1.26	8
Extract % (irrigation)	1st der	0.60	0.63	0.88	0.60	0.0975	1.54	5
TN % (dry land)	SNV	0.10	0.84	0.11	0.75	0.0010	2.01	10
TN % (irrigation)	1st der	0.09	0.60	0.10	0.78	-0.0530	1.88	6
TSN % (dry land)	1st der & SNV	0.06	0.75	0.06	0.71	-0.0008	1.84	8
TSN % (irrigation)	SNV	0.06	0.45	0.08	0.50	-0.0393	1.39	6
KI (dry land)	Mean norm	2.48	0.46	4.07	0.46	0.1920	1.13	15
KI (irrigation)	SNV	2.21	0.71	3.40	0.48	-0.8790	1.38	10
FAN mg/L (dry land)	SNV	18.05	0.75	28.37	0.77	3.3580	1.56	16
FAN mg/L (irrigation)	None	19.43	0.59	29.05	0.63	-11.7890	1.54	10
DP W.K. (dry land)	SNV	58.62	0.73	59.42	0.72	-13.108	1.89	11
DP W.K. (irrigation)	1st der & SNV	51.20	0.54	84.30	0.40	18.2450	1.27	6
Viscosity cP(dry land)	2nd der	0.01	0.62	0.02	0.26	-0.0073	1.38	10
Viscosity cP (irrigation)	1st der	0.01	0.62	0.02	0.40	-0.0087	1.67	10
AAL (dry land)	Mean norm	0.39	0.39	1.73	0.20	0.2190	1.11	7
AAL (irrigation)	None	2.10	0.28	1.73	0.22	-0.2330	1.10	4
β-glucans mg/L (dry land)	2nd der	22.32	0.61	55.19	0.46	-24.3761	1.30	11
β-glucans mg/L (irrigation)	1st der & SNV	26.73	0.57	29.83	0.61	-5.2311	3.79	6

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC=standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; mean norm=mean normalization; none=no spectral pre-treatment

Table 3.12 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRFlex N-500 data (The Unscrambler software) for dry land whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	None	0.70	0.47	0.74		0.41	0.0014	1.27	6
	Test set	None	0.70	0.49		0.71	0.43	-0.1349	1.33	6
Plumpness (%)	CV	SNV	2.83	0.44	2.94		0.40	-0.0027	1.28	9
	Test set	SNV	2.77	0.47		3.03	0.36	0.2008	1.24	3
Extract (%)	CV	none	0.70	0.58	0.76		0.51	0.0021	1.27	9
	Test set	none	0.74	0.57		0.75	0.40	0.1180	1.30	9
TN (%)	CV	SNV	0.11	0.81	0.11		0.78	-0.0002	1.91	7
	Test set	SNV	0.11	0.82		0.11	0.76	0.0061	1.97	7
TSN (%)	CV	1st der	0.06	0.71	0.07		0.66	0.0001	1.72	7
	Test set	1st der	0.06	0.70		0.06	0.73	0.0010	1.90	6
KI	CV	none	3.65	0.09	3.71		0.06	-0.0037	1.24	2
	Test set	none	3.25	0.07		4.37	0.11	0.5090	1.05	1
FAN (mg/L)	CV	SNV	24.07	0.64	26.03		0.64	-0.0290	1.70	9
	Test set	SNV	24.23	0.59		25.84	0.68	5.5080	1.71	8
DP (W.K.)	CV	none	62.11	0.70	66.75		0.65	-0.034	1.69	10
	Test set	none	63.91	0.68		59.70	0.72	-8.788	1.89	9
Viscosity (cP)	CV	2nd der	0.02	0.33	0.02		0.28	-0.00002	1.51	4
	Test set	2nd der	0.02	0.34		0.02	0.26	-0.0078	1.38	3
AAL	CV	mean norm	1.72	0.33	1.78		0.28	-0.0044	1.08	4
	Test set	mean norm	1.78	0.33		1.72	0.20	0.2630	1.11	3
β-glucans (mg/L)	CV	none	15.86	0.06	16.06		0.04	-0.0002	4.46	1
	Test set	none	15.86	0.06		24.75	0.13	-12.5968	2.90	1

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC=standard error of calibration; SEP=standard error of prediction; SECV=standard error of cross-validation; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; mean norm=mean normalization; none=no spectral pre-treatment

Table 3.13 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRFlex N-500 data (The Unscrambler software) for irrigation whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.60	0.16	0.63		0.08	0.0056	1.04	3
	Test set	none	0.61	0.13		0.62	0.12	-0.0317	1.06	2
Plumpness (%)	CV	mean norm	3.64	0.03	3.66		0.02	0.0020	1.23	1
	Test set	mean norm	3.62	0.03		3.47	0.07	-0.0962	1.29	2
Extract (%)	CV	2^{nd} der	0.70	0.62	0.77		0.54	0.0041	1.77	4
	Test set	2^{nd} der	0.67	0.55		0.80	0.69	-0.0065	1.70	4
TN (%)	CV	1^{st} der	0.09	0.72	0.10		0.67	-0.0016	1.99	5
	Test set	1^{st} der	0.09	0.60		0.09	0.85	-0.0457	2.13	5
TSN (%)	CV	None	0.07	0.61	0.07		0.51	-0.0002	1.56	8
	Test set	None	0.06	0.59		0.08	0.47	-0.0230	1.38	7
KI	CV	None	2.63	0.62	2.97		0.52	0.0280	1.58	8
	Test set	None	2.53	0.61		3.02	0.59	-0.0819	1.50	8
FAN (mg/L)	CV	SNV	20.30	0.60	22.13		0.53	-0.0247	2.03	6
	Test set	SNV	19.79	0.58		21.97	0.63	-6.3984	2.04	6
DP (W.K.)	CV	none	62.38	0.50	68.91		0.39	0.2750	1.55	8
	Test set	none	62.56	0.32		85.95	0.40	28.1070	1.24	6
Viscosity (cP)	CV	none	0.02	0.13	0.02		0.09	-0.0001	1.57	1
	Test set	none	0.02	0.18		0.03	0.07	-0.0092	1.23	1
AAL	CV	none	1.71	0.46	1.89		0.35	-0.0054	1.01	8
	Test set	none	2.21	0.23		1.70	0.22	-0.3878	1.12	4
β-glucans (mg/L)	CV	1^{st} der	27.72	0.58	31.35		0.47	-0.6030	3.60	5
	Test set	1^{st} der	30.27	0.45		38.58	0.36	-2.5600	2.93	4

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1^{st} der=Savitzky-Golay first derivative, 9 points; 2^{nd} der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3.14 Summary of calibration and validation results for the best test set validation models from Bruker MPA data

Property	Pretreatment	Calibration		Validation				
		RMSEE	R^2	RMSEP	r^2	Bias	RPD	PLS
Moisture % (dry land)	Normal	0.66	0.56	0.63	0.53	0.0771	1.46	6
Moisture % (irrigation)	SNV	0.55	0.29	0.58	0.16	0.0011	1.07	3
Plumpness % (dry land)	None	2.93	0.39	2.76	0.29	0.0818	1.18	13
Plumpness % (irrigation)	None	2.08	0.60	2.31	0.49	-0.1730	1.38	7
Extract % (dry land)	None	0.92	0.35	0.85	0.24	-0.0788	1.14	5
Extract % (irrigation)	SNV	0.57	0.69	0.93	0.55	-0.0698	1.45	10
TN % (dry land)	Normal	0.09	0.90	0.13	0.64	-0.0086	1.65	13
TN % (irrigation)	SNV	0.07	0.79	0.12	0.70	0.0550	1.79	12
TSN % (dry land)	None	0.05	0.81	0.09	0.44	-0.0086	1.32	13
TSN % (irrigation)	SNV	0.05	0.70	0.10	0.30	0.0302	1.20	10
KI (dry land)	1^{st} der & MSC	2.76	0.26	4.43	0.09	-0.628	1.05	4
KI (irrigation)	Normal	3.05	0.47	4.05	0.27	0.5160	1.16	8
FAN mg/L (dry land)	None	21.80	0.69	34.80	0.39	-6.170	1.28	14
FAN mg/L (irrigation)	SNV	17.20	0.69	35.30	0.38	4.2100	1.27	10
DP W.K. (dry land)	None	53.80	0.78	74.30	0.59	9.800	1.53	13
DP W.K. (irrigation)	Normal	42.70	0.71	94.70	0.22	-7.5400	1.13	11
Viscosity cP(dry land)	SNV	0.02	0.23	0.02	0.12	0.007	1.07	3
Viscosity cP (irrigation)	None	0.02	0.27	0.03	0.14	0.0097	1.08	4
AAL (dry land)	Normal	1.81	0.25	1.67	0.17	-0.237	1.09	2
AAL (irrigation)	Normal	2.35	0.15	1.96	0.05	0.2190	0.97	3
β-glucans mg/L(dry land)	SNV	16.60	0.02	19.10	0.07	5.680	1.02	1
β-glucans mg /L(irrigation)	1^{st} der & MSC	39.30	0.22	37.50	0.43	-1.4000	1.27	3

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); RMSEE=root mean square error of estimation; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; MSC=multiplicative scatter correction; 1^{st} der=Savitzky-Golay first derivative, 9 points; 2^{nd} der=Savitzky-Golay second derivative,17 points; Normal=min max normalization; none=no spectral pre-treatment

Table 3.15 Summary of calibration and validation results for the best test set validation models from Büchi NIRLab N-200 data for whole grain and flour samples

Sample	Property	Pretreatment	Calibration		Validation				
			SEC	R^2	SEP	r^2	Bias	RPD	PLS
Whole grain	Moisture % (dry land)	2nd der & SNV	0.73	0.43	0.69	0.47	0.0514	1.36	2
	Moisture % (irrigation)	1st der	0.58	0.07	0.63	0.08	-0.0491	1.00	4
Flour	Moisture % (dry land)	None	0.46	0.21	0.46	0.76	0.0172	2.03	6
	Moisture % (irrigation)	None	0.29	0.80	0.35	0.69	0.0488	1.81	6
Whole grain	Plumpness %(dry land)	2nd der & SNV	1.96	0.71	3.18	0.36	0.5059	1.27	8
	Plumpness % (irrigation)	1st der & SNV	3.50	0.58	3.10	0.52	-1.3061	1.23	3
Flour	Plumpness %(dry land)	1st der	2.08	0.67	3.06	0.34	-0.3157	1.32	8
	Plumpness % (irrigation)	1st der	3.46	0.59	2.91	0.50	-1.3335	1.32	5
Whole grain	Extract %(dry land)	2nd der & SNV	0.73	0.64	0.71	0.55	0.0676	1.49	5
	Extract % (irrigation)	2nd der	1.20	0.05	0.87	0.34	0.0719	1.12	1
Flour	Extract % (dry land)	1st der	0.58	0.76	0.81	0.48	0.1777	1.32	3
	Extract % (irrigation)	None	0.42	0.89	0.72	0.55	0.0911	1.36	10
Whole grain	TN % (dry land)	2nd der & SNV	0.11	0.83	0.11	0.79	0.0073	2.18	5
	TN % (irrigation)	2nd der	0.10	0.68	0.15	0.27	0.0252	1.06	5
Flour	TN % (dry land)	SNV	0.10	0.86	0.09	0.84	-0.0130	2.51	7
	TN % (irrigation)	1st der	0.09	0.78	0.10	0.65	-0.0093	1.68	5
Whole grain	TSN % (dry land)	2nd der	0.04	0.87	0.07	0.55	0.0104	1.47	8
	TSN % (irrigation)	None	0.11	0.11	0.10	0.03	0.0114	0.97	1
Flour	TSN % (dry land)	2nd der & SNV	0.07	0.68	0.07	0.59	0.0003	1.56	2
	TSN % (irrigation)	Mean norm	0.08	0.35	0.06	0.62	-0.0025	1.61	6
Whole grain	KI (dry land)	SNV	3.43	0.18	3.11	0.18	0.2215	1.09	5
	KI (irrigation)	None	4.03	0.098	3.32	0.11	0.6046	1.06	1
Flour	KI (dry land)	SNV	3.20	0.29	3.15	0.20	0.3165	1.08	6
	KI (irrigation)	2nd der & SNV	3.26	0.41	2.74	0.39	-0.1803	1.28	3
Whole grain	FAN mg/L (dry land)	SNV	34.31	0.18	26.15	0.36	1.5392	1.25	4
	FAN mg/L (irrigation)	None	35.05	0.096	30.87	0.13	-0.1981	1.07	2
Flour	FAN mg/L (dry land)	1st der	29.82	0.42	21.03	0.60	5.1192	1.56	3
	FAN mg/L (irrigation)	Mean norm	5.40	0.54	22.86	0.54	-1.1544	1.45	13
Whole grain	DP W.K. (dry land)	2nd der & SNV	68.30	0.68	70.74	0.56	0.5031	1.48	4
	DP W.K. (irrigation)	None	70.98	0.378	71.90	0.15	-5.6767	1.06	9
Flour	DP W.K. (dry land)	1st der	73.21	0.61	79.07	0.47	-0.0347	1.34	4
	DP W.K. (irrigation)	Mean norm	47.71	0.67	49.28	0.58	5.5782	1.54	7
Whole grain	Viscosity cP (dry land)	2nd der & SNV	0.02	0.44	0.02	0.34	0.0023	1.21	4
	Viscosity cP (irrigation)	1st der & SNV	0.03	0.044	0.02	0.25	0.0015	1.12	1
Flour	Viscosity cP (dry land)	2nd der	0.02	0.55	0.02	0.43	0.0029	1.22	5
	Viscosity cP (irrigation)	None	0.01	0.59	0.02	0.47	-0.0021	1.37	10
Whole grain	AAL (dry land)	Mean norm	1.29	0.62	1.60	0.25	0.0242	1.13	10
	AAL (irrigation)	None	1.54	0.50	1.78	0.20	0.2557	1.07	9
Flour	AAL (dry land)	2nd der	1.51	0.47	1.58	0.25	0.0773	1.14	4
	AAL (irrigation)	Mean norm	1.92	0.32	1.70	0.23	-0.4187	1.12	7
Whole grain	β-glucan mg/L (dry land)	1st der	14.35	0.17	15.45	0.29	-3.9807	1.86	4
	β-glucan mg/L (irrigation)	2nd der & SNV	45.68	0.11	44.14	0.23	-2.2988	1.66	1
Flour	β-glucan mg/L (dry land)	Mean norm	14.99	0.23	15.69	0.25	0.0913	1.83	5
	β-glucan mg/L (irrigation)	1st der	35.07	0.47	38.39	0.42	-3.0195	1.90	4

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC=standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; mean norm=mean normalization; none=no spectral pre-treatment

Table 3.16 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for dry land whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	2nd der	0.59	0.61	0.63		0.55	-0.0022	1.46	3
	Test set	2nd der	0.62	0.59		0.59	0.60	0.0462	1.58	3
Plumpness (%)	CV	none	3.01	0.34	3.21		0.26	-0.0035	1.26	6
	Test set	none	2.97	0.33		3.34	0.33	0.3840	1.21	5
Extract (%)	CV	2nd der	0.69	0.65	0.74		0.60	0.0039	1.43	3
	Test set	2nd der	0.71	0.67		0.67	0.62	0.0082	1.59	3
TN (%)	CV	2nd der	0.10	0.84	0.11		0.81	0.0012	2.11	4
	Test set	2nd der	0.11	0.83		0.10	0.81	0.0131	2.26	3
TSN (%)	CV	1st der	0.06	0.75	0.07		0.64	-0.0003	1.51	7
	Test set	1st der	0.07	0.68		0.07	0.53	-0.0003	1.46	7
KI	CV	mean norm	3.47	0.09	3.57		0.05	0.0014	0.95	1
	Test set	mean norm	3.63	0.07		3.23	0.10	0.1057	1.05	1
FAN (mg/L)	CV	SNV	24.87	0.53	26.98		0.45	-0.1836	1.21	6
	Test set	SNV	26.44	0.52		21.93	0.52	8.9617	1.49	5
DP (W.K.)	CV	2nd der	71.06	0.62	75.09		0.71	-0.0419	1.41	3
	Test set	2nd der	72.77	0.63		69.70	0.73	-1.8026	1.52	3
Viscosity (cP)	CV	2nd der	0.02	0.35	0.02		0.32	-0.0001	1.07	1
	Test set	2nd der	0.02	0.37		0.02	0.35	0.0019	1.22	1
AAL	CV	mean norm	1.34	0.55	1.50		0.45	-0.0081	1.20	7
	Test set	Mean norm	1.62	0.40		1.65	0.22	-0.0026	1.09	3
β-glucans (mg/L)	CV	1st der	14.66	0.22	15.21		0.16	0.1297	1.89	3
	Test set	1st der	15.19	0.07		16.35	0.24	-2.9668	1.76	2

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3.17 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for irrigation whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	1st der	0.55	0.13	0.62		0.13	-0.0045	1.01	4
	Test set	1st der	0.42	0.07		0.66	0.07	-0.0825	0.94	4
Plumpness	CV	1st der	2.96	0.65	3.14		0.60	0.0347	1.22	4
(%)	Test set	1st der	3.04	0.68		3.00	0.56	-0.8338	1.28	4
Extract (%)	CV	2nd der	1.10	0.09	1.13		0.04	-0.0043	0.86	1
	Test set	2nd der	1.20	0.05		0.87	0.34	0.0755	1.12	1
TN (%)	CV	2nd der	0.13	0.38	0.14		0.38	0.00001	1.16	2
	Test set	2nd der	0.13	0.46		0.17	0.10	0.0147	0.94	2
TSN (%)	CV	1st der	0.10	0.23	0.11		0.10	-0.0002	0.91	3
	Test set	1st der	0.11	0.16		0.10	0.04	0.0159	1.01	1
KI	CV	none	3.82	0.093	3.88		0.07	-0.0080	0.90	1
	Test set	none	4.03	0.098		3.32	0.11	0.6046	1.06	1
FAN (mg/L)	CV	2nd der	32.82	0.189	33.84		0.14	-0.0214	0.98	1
	Test set	2nd der	33.55	0.224		31.49	0.22	-6.2397	1.05	1
DP (W.K.)	CV	none	85.82	0.001	88.50		0.19	-0.7112	0.86	1
	Test set	none	70.98	0.378		71.90	0.15	-5.6767	1.06	1
Viscosity	CV	mean norm	0.03	0.005	0.03		0.04	0.0000	0.79	1
(cP)	Test set	mean norm	0.02	0.07		0.02	0.29	0.0030	1.08	1
AAL	CV	SNV	1.95	0.23	2.14		0.10	0.0152	0.89	5
	Test set	SNV	2.25	0.09		1.85	0.07	-0.0750	1.03	4
β-glucans	CV	2nd der	46.74	0.07	47.87		0.03	0.2320	1.53	1
(mg/L)	Test set	2nd der	47.75	0.01		46.57	0.30	-4.3962	1.57	1

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3.18 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for dry land flour samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	mean norm	0.86	0.73	0.49		0.72	-0.0010	1.88	2
	Test set	mean norm	0.47	0.74		0.51	0.71	0.0197	1.82	2
Plumpness	CV	1st der	2.53	0.51	2.84		0.39	-0.0274	1.42	7
(%)	Test set	1st der	2.56	0.50		2.75	0.45	0.2029	1.47	7
Extract (%)	CV	mean norm	0.81	0.52	0.84		0.48	-0.0048	1.27	2
	Test set	mean norm	0.81	0.56		0.82	0.43	-0.0031	1.30	2
TN (%)	CV	SNV	0.10	0.84	0.11		0.82	0.0003	2.17	5
	Test set	SNV	0.11	0.83		0.09	0.84	-0.0237	2.46	4
TSN (%)	CV	2nd der	0.06	0.68	0.07		0.65	0.0001	1.58	3
	Test set	2nd der	0.06	0.72		0.07	0.61	0.0048	1.59	3
KI	CV	SNV	3.19	0.24	3.39		0.15	0.0206	1.00	4
	Test set	SNV	3.37	0.21		3.07	0.20	0.3861	1.11	3
FAN (mg/L)	CV	1st der	26.92	0.47	28.17		0.53	-0.1367	1.16	3
	Test set	1st der	29.13	0.45		21.23	0.58	5.8125	1.54	3
DP (W.K.)	CV	SNV	73.89	0.58	78.21		0.53	0.0127	1.35	5
	Test set	SNV	72.98	0.62		77.35	0.49	-10.1074	1.37	5
Viscosity	CV	2nd der	0.41	0.41	0.02		0.59	0.0001	1.09	3
(cP)	Test set	2nd der	0.38	0.38		0.02	0.65	0.0039	1.30	2
AAL	CV	2nd der	1.65	0.31	1.73		0.25	0.0032	1.04	2
	Test set	2nd der	1.60	0.41		1.55	0.26	0.0652	1.16	2
β-glucans	CV	SNV	15.00	0.32	15.39		0.25	0.0228	1.87	5
(mg/L)	Test set	SNV	15.36	0.30		14.64	0.35	1.6123	1.96	5

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der= Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3.19 Summary of best calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for irrigation flour samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	mean norm	0.32	0.75	0.34		0.72	-0.0013	1.84	5
	Test set	mean norm	0.30	0.79		0.36	0.68	0.0430	1.76	5
Plumpness	CV	none	4.11	0.35	4.29		0.30	0.0270	0.90	3
(%)	Test set	none	4.47	0.35		3.05	0.40	-2.2555	1.26	3
Extract (%)	CV	none	0.53	0.79	0.63		0.71	0.0158	1.56	8
	Test set	none	0.55	0.81		0.66	0.58	0.1184	1.49	7
TN (%)	CV	1st der	0.10	0.67	0.11		0.64	-0.0007	1.50	3
	Test set	1st der	0.10	0.69		0.10	0.61	-0.0056	1.60	3
TSN (%)	CV	mean norm	0.43	0.43	0.08		0.38	0.0001	1.30	6
	Test set	mean norm	0.34	0.34		0.06	0.59	-0.0048	1.54	4
KI	CV	1st der	3.51	0.24	3.69		0.16	0.0175	0.95	2
	Test set	1st der	3.83	0.20		2.81	0.34	-0.4112	1.25	2
FAN (mg/L)	CV	SNV	21.84	0.62	23.73		0.55	0.0054	1.39	6
	Test set	SNV	21.26	0.66		23.78	0.49	-4.3789	1.39	6
DP (W.K.)	CV	mean norm	47.34	0.65	55.51		0.52	-0.4862	1.37	7
	Test set	mean norm	61.05	0.45		55.92	0.44	2.7709	1.34	3
Viscosity	CV	none	0.02	0.44	0.02		0.28	0.0001	1.13	6
(cP)	Test set	none	0.02	0.35		0.02	0.44	-0.0016	1.32	5
AAL	CV	mean norm	1.85	0.29	2.16		0.10	-0.0190	0.89	7
	Test set	mean norm	2.05	0.23		1.74	0.18	-0.3574	1.10	4
β-glucans	CV	1st der	31.89	0.57	36.51		0.44	-0.9815	2.00	6
(mg/L)	Test set	1st der	34.81	0.48		33.83	0.54	-3.8780	2.16	6

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3.20 Summary of calibration and validation results for test set validation models from the Büchi NIRLab N-200 data for best cross validation results of moisture content, TN and TSN

Property	Samples	Pretreatment	Calibration		Validation				
			SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture (%)	Dry land whole grain	none	0.59	0.58	0.69	0.38	-0.0359	1.25	15
	Dry land flour	2nd der	0.52	0.66	0.59	0.54	-0.0265	1.46	4
	Irrigation whole grain	2nd der + SNV	0.52	0.37	0.65	0.02	0.0557	0.89	4
	Irrigation flour	none	0.51	0.35	0.40	0.60	0.1082	1.46	4
TN (%)	Dry land whole grain	SNV	0.17	0.72	0.20	0.61	0.0073	1.58	13
	Dry land flour	None	0.20	0.62	0.20	0.57	-0.0044	1.52	4
	Irrigation whole grain	Mean norm	0.14	0.50	0.14	0.37	0.0062	1.24	8
	Irrigation flour	2nd der + SNV	0.13	0.58	0.11	0.60	0.0068	1.58	2
TSN (%)	Dry land whole grain	1st der + SNV	0.10	0.65	0.10	0.59	-0.0032	1.52	9
	Dry land flour	SNV	0.13	0.43	0.12	0.37	0.0070	1.25	2
	Irrigation whole grain	Mean norm	0.11	0.33	0.09	0.43	0.0018	1.23	4
	Irrigation flour	Mean norm	0.09	0.49	0.09	0.40	-0.0099	1.27	5

TN=total nitrogen; TSN=total soluble nitrogen; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC=standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; mean norm=mean normalization; none=no spectral pre-treatment

Table 3.21 Summary of calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data (The Unscrambler software) for dry land whole grain samples

Property	Validation	Pretreatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	None	0.62	0.52	0.65		0.47	-0.0025	1.33	8
	Test set	None	0.62	0.53		0.62	0.49	-0.0774	1.39	8
TN (%)	CV	SNV	0.17	0.73	0.20		0.61	0.0017	1.52	14
	Test set	SNV	0.18	0.70		0.24	0.44	-0.0100	1.27	14
TSN (%)	CV	1st der + SNV	0.11	0.58	0.11		0.53	-0.0005	1.39	5
	Test set	1st der + SNV	0.13	0.41		0.13	0.33	0.0047	1.22	5

TN=total nitrogen; TSN=total soluble nitrogen; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; SEP=standard error of prediction; r^2=coefficient of determination (validation); RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; none=no spectral pre-treatment

Table 3.22 Summary of calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data (The Unscrambler software) for dry land flour samples

Property	Validation	Pretreatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	2nd der	0.55	0.62	0.57		0.59	-0.0010	1.53	4
	Test set	2nd der	0.53	0.65		0.59	0.55	-0.0288	1.48	4
TN (%)	CV	None	0.20	0.63	0.20		0.61	0.0009	1.53	5
	Test set	None	0.19	0.66		0.20	0.56	0.0020	1.50	5
TSN (%)	CV	SNV	0.11	0.54	0.12		0.50	0.0004	1.35	6
	Test set	SNV	0.12	0.53		0.11	0.53	0.0189	1.44	6

TN=total nitrogen; TSN=total soluble nitrogen; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 2nd der=Savitzky-Golay second derivative, 17 points; none=no spectral pre-treatment

Table 3.23 Summary of calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data (The Unscrambler software) for irrigation whole grain samples

Property	Validation	Pretreatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	2nd der + SNV	0.54	0.28	0.57		0.20	-0.0011	1.03	4
	Test set	2nd der + SNV	0.52	0.37		0.60	0.11	0.0319	0.97	4
TN (%)	CV	Mean norm	0.13	0.53	0.14		0.47	0.0003	1.27	6
	Test set	Mean norm	0.14	0.53		0.13	0.49	0.0020	1.38	6
TSN (%)	CV	Mean norm	0.09	0.54	0.09		0.48	-0.00004	1.23	5
	Test set	Mean norm	0.09	0.55		0.09	0.50	-0.0059	1.31	5

TN=total nitrogen; TSN=total soluble nitrogen; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 2nd der=Savitzky-Golay second derivative, 17 points; mean norm=mean normalization

Table 3.24 Summary of calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data (The Unscrambler software) for irrigation flour samples

Property	Validation	Pretreatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	None	0.49	0.39	0.51		0.34	-0.0001	1.14	4
	Test set	None	0.50	0.42		0.47	0.37	0.0284	1.24	4
TN (%)	CV	2nd der + SNV	0.13	0.57	0.13		0.55	0.0002	1.39	1
	Test set	2nd der + SNV	0.13	0.56		0.11	0.59	0.0046	1.57	1
TSN (%)	CV	Mean norm	0.07	0.66	0.08		0.52	-0.0002	1.35	11
	Test set	Mean norm	0.07	0.64		0.09	0.43	-0.0131	1.26	11

TN=total nitrogen; TSN=total soluble nitrogen; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; SNV=standard normal variate; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Moisture content

A fairly poor moisture content prediction model was developed from the Büchi NIRFlex N-500 data (**Table 3.11**) for dry land whole grain samples with an r^2 of 0.48, SEP of 0.69% and low RPD of 1.36. Variable selection, using uncertainty testing in The Unscrambler (**Table 3.12**) delivered similarly poor results (r^2 = 0.43, SEP = 0.71%, RPD = 1.33). Irrigation whole grain models (**Table 3.11**) delivered an r^2 of 0.19, SEP of 0.60% and a low RPD of 1.10, which is not recommended for use as indicated by the extremely poor r^2 and RPD values. The process of variable selection for irrigation samples (**Table 3.13**) was unsuccessful (r^2 = 0.12, SEP = 0.62%, RPD = 1.06).

Calibration models developed from the Bruker MPA data (**Table 3.14**) for dry land samples (r^2 = 0.53, SEP = 0.63%, RPD = 1.46) were acceptable for rough screening, given that more than 50% of the variance in the NIR data is accounted for by the variance in the reference data (Williams, 2001). Moisture content prediction models for irrigation samples with this instrument were unacceptable (r^2 = 0.16, SEP = 0.58%, RPD = 1.06).

For the Büchi NIRLab N-200 data (**Table 3.15**), a relatively poor correlation was obtained for dry land whole grain samples (r^2 = 0.47, SEP = 0.69%, RPD = 1.36). Variable selection (**Table 3.16**) improved the model (r^2 = 0.60, SEP =0.59%, RPD = 1.58) to a level appropriate for rough screening. Extremely poor results were obtained for irrigation whole grain samples when using the entire spectral region (r^2 = 0.08, SEP = 0.63%, RPD = 1.00) or selected variables (r^2 = 0.07, SEP = 0.66%, RPD = 0.94, **Table 3.17**).

Good dry land sample models were obtained for the Büchi NIRLab N-200 flour data (**Table 3.15**), with an r^2 of 0.76, SEP of 0.46% and RPD of 2.03, which indicated that this calibration could be used for screening purposes. The application of selected variables to dry land flour samples (**Table 3.18**) proved acceptable for screening purposes (r^2 = 0.71, SEP = 0.51%, RPD = 1.82). Irrigation flour sample calibration models (r^2 = 0.69, SEP = 0.35%, RPD = 1.81, **Table 3.15**) were also acceptable for screening purposes; similar results were obtained for variable selection (r^2 = 0.68, SEP = 0.36%, RPD = 1.76; **Table 3.19**).

For the combined 2008 and 2009 samples from the Büchi NIRLab N-200 (**Table 3.20**), test set validation of dry land whole grain samples delivered extremely poor results (r^2 = 0.38, SEP = 0.69%, RPD = 1.25) and the selection of significant wavelengths (**Table 3.21**) did not show much improvement (r^2 = 0.49, SEP = 0.62, RPD = 1.39). Results for irrigation whole grain samples (**Table 3.20**, **3.23**) were extremely disappointing (r^2 = 0.02, SEP = 0.40%, RPD = 0.89), and variable selection (r^2 = 0.11, SEP = 0.60, RPD = 0.97) showed no improvement. More acceptable results were obtained for dry land flour samples (**Table 3.20**) (r^2 = 0.54, SEP = 0.59%, RPD = 1.46) but calibrations were only good enough for rough screening. The use of selected spectral variables (**Table 3.22**) delivered results acceptable for rough screening purposes (r^2 = 0.55, SEP = 0.59, RPD = 1.48). Results appropriate for screening were obtained for irrigation flour samples (r^2 = 0.60, SEP = 0.40%, RPD = 1.46; **Table 3.20**), while variable selection (**Table 3.24**) delivered unacceptable results (r^2 = 0.37, SEP = 0.47, RPD = 1.24).

Plumpness

The model developed for dry land whole grain samples with the Büchi NIRFlex N-500 data (**Table 3.11**) delivered very poor results (r^2 = 0.37, SEP = 3.03%, RPD = 1.25) while the application of uncertainty testing for spectral variable selection (**Table 3.12**) did not show a great deal of improvement (r^2 = 0.36, SEP = 3.03%, RPD = 1.24). Models developed for irrigation whole grain samples (**Table 3.11**) delivered an r^2 of 0.07, SEP of 3.47% and RPD of 1.29, while variable selection (**Table 3.13**) delivered similar results (r^2 = 0.07, SEP = 3.47%, RPD = 1.29). These irrigation whole grain models were exceptionally poor and unacceptable for prediction purposes.

Calibrations developed with the Bruker MPA data (**Table 3.14**) also delivered poor results. Dry land whole grain (r^2 = 0.29, SEP = 2.76%, RPD = 1.18) and irrigation whole grain sample models (r^2 = 0.49, SEP = 2.31%, RPD = 1.38) were not acceptable for screening purposes

Büchi NIRLab N-200 dry land whole grain spectra (**Table 3.15**) delivered a very poor correlation and would not be acceptable for use even for rough screening purposes (r^2 = 0.36, SEP = 3.18%, RPD = 1.27). When evaluated with uncertainty testing (**Table 3.16**), the results (r^2 = 0.33, SEP = 3.34%, RPD = 1.21) were still unacceptable. The whole grain irrigation sample model (r^2 = 0.52, SEP = 3.10%, RPD = 1.23; **Table 3.15**) could be used for rough screening purposes; similar results were obtained with variable selection (**Table 3.17**), with an r^2 of 0.56, SEP of 3.00% and RPD of 1.28. This irrigation whole grain sample model, developed with only significant wavelengths, could also be used for rough screening purposes in the earlier stages of the breeding programme.

Dry land flour sample prediction (**Table 3.15**) with the Büchi NIRLab N-200 delivered a poor calibration (r^2 = 0.34, SEP = 3.06%, RPD = 1.32) while uncertainty testing variable selection (**Table 3.18**) also delivered relatively poor results (r^2 = 0.45, SEP = 2.75%, RPD = 1.47). More acceptable results were only obtained for irrigation flour samples (**Table 3.15**); 50% of the variation in the NIR data is accounted for by the variation in the reference data (r^2 = 0.50, SEP = 2.91%, RPD = 1.32) which indicates that the calibration would be acceptable for rough screening purposes. The process of variable selection (r^2 = 0.40, SEP = 3.05%, RPD = 1.26; **Table 3.20**) delivered very poor results.

Extract

A poor correlation was observed for dry land whole grain sample data obtained with the Büchi NIRFlex N-500 instrument (r^2 = 0.39, SEP = 0.77%, RPD = 1.26; **Table 3.11**) and variable selection (**Table 3.12**) delivered similarly poor results (r^2 = 0.40, SEP = 0.75%, RPD = 1.30) which could not be used in future predictions. An acceptable calibration was obtained for predicting extract from irrigation whole grain samples (**Table 3.11**) with an r^2 of 0.60, SEP of 0.88% and RPD of 1.54, which would be acceptable for rough screening purposes. The use of selected variables (**Table 3.13**) delivered good results (r^2 = 0.69, SEP = 0.80% and RPD = 1.70) which could be used for screening.

For the Bruker MPA (**Table 3.14**) dry land data (r^2 = 0.24, SEP = 0.85% and RPD = 1.14), a poor correlation was obtained, but the irrigation whole grain sample model (r^2 = 0.55, SEP = 0.93%, RPD = 1.45) was acceptable for rough screening purposes.

The Büchi NIRLab N-200 whole grain dry land samples (**Table 3.15**) delivered results (r^2 = 0.55, SEP = 0.71%, RPD = 1.49) acceptable for rough screening, which were improved with the selection of significant wavelengths (r^2 = 0.62, SEP = 0.67%, RPD = 1.59; **Table 3.16**). However, poor results were obtained for irrigation whole grain samples (r^2 = 0.34, SEP = 0.87%, RPD = 1.12; **Table 3.15**) even after variable selection (**Table 3.17**) (r^2 = 0.34, SEP = 0.87%, RPD = 1.12).

Dry land flour models developed with Buchi NIRLab N-200 data (r^2 = 0.48, SEP = 0.81%, RPD = 1.32, **Table 3.15)** and variable selection (**Table 3.18**) (r^2 = 0.43, SEP = 0.82%, RPD = 1.30) showed a poor correlation. Acceptable results were obtained for irrigation flour samples (r^2 = 0.55, SEP = 0.72%, RPD = 1.36; **Table 3.15**). An improved model (r^2 = 0.58, SEP = 0.66%, RPD = 1.49) was obtained with variable selection (**Table 3.19**) and would be acceptable for rough screening purposes.

Total nitrogen

Results for prediction of TN from the dry land whole grain sample Büchi NIRFlex N-500 data (r^2 = 0.75, SEP = 0.11%, RPD = 2.01; **Table 3.11**) were acceptable for screening purposes as indicated by the relatively high RPD and low SEP. The application of variable selection to this data set (**Table 3.12**) delivered a very good model (r^2 = 0.76, SEP = 0.11%, RPD = 1.97), also acceptable for screening. A very good calibration was also developed for irrigation whole grain sample Büchi NIRFlex N-500 data (r^2 = 0.78, SEP = 0.10%, RPD = 1.88, **Table 3.11**) and with variable selection (**Table 3.13**) a calibration was obtained which could be used for almost any application (r^2 = 0.85, SEP = 0.09%, RPD = 2.13).

For the Bruker MPA data (**Table 3.14**), dry land whole grain sample calibration results (r^2 = 0.64, SEP = 0.13%, RPD = 1.65) would only be acceptable for rough screening at earlier stages of the breeding programme. Irrigation sample calibrations delivered a r^2 of 0.70, SEP of 0.12% and an RPD of 1.79 which would be acceptable for screening purposes.

The model developed with the Büchi NIRLab N-200 whole grain dry land samples (r^2 = 0.79, SEP = 0.11%, RPD = 2.18; **Table 3.15**) proved to be acceptable for screening purposes while similar results were obtained with variable selection (r^2 = 0.81, SEP = 0.10%, RPD = 2.26; **Table 3.16**). Irrigation whole grain sample results (**Table 3.15**) were unacceptable when using the entire spectral region (r^2 = 0.27, SEP = 0.15%, RPD = 1.06) as well as selected variables (r^2 = 0.10, SEP = 0.17%, RPD = 0.94).

Büchi NIRLab N-200 dry land flour data delivered very good results (r^2 = 0.84, SEP = 0.09%, RPD 2.51; **Table 3.15**) where the RPD was higher than most other calibrations obtained in this study, indicating a good sample range. The use of selected variables delivered very good results for dry land flour samples (**Table 3.18**) where the test set validation (r^2 = 0.84, SEP = 0.09%, RPD

= 2.46) model could be used with caution in most applications. An acceptable calibration was developed for irrigation flour samples (R^2 = 0.65, SEP = 0.10%, RPD = 1.68; **Table 3.15**) and for variable selection (**Table 3.19**), the test set validated model (r^2 = 0.61, SEP = 0.10%, RPD = 1.60) also delivered results acceptable for rough screening.

For the combined samples from the 2008 and 2009 harvest seasons, test set validation for dry land whole grain samples (**Table 3.20**) delivered good results (r^2 = 0.61, SEP = 0.20%, RPD = 1.58) that could be used for rough screening, but variable selection (**Table 3.21**) was unsuccessful (r^2 = 0.44, SEP = 0.24%, RPD = 1.27). Irrigation whole grain samples (**Table 3.20**) delivered very poor results (r^2 = 0.37, SEP = 0.14%, RPD = 1.24) while the use of selected variables (**Table 3.23**) (r^2 = 0.49, SEP = 0.13%, RPD = 1.38) showed no improvement. The model developed for dry land flour samples (**Table 3.20**) was acceptable for rough screening (r^2 = 0.57, SEP = 0.20%, RPD = 1.52) and similar results were obtained in the case of variable selection (r^2 = 0.56, SEP = 0.20, RPD = 1.50; **Table 3.22**). The models for prediction of TN from irrigation flour samples delivered results acceptable for rough screening when using the entire spectral region (r^2 = 0.60, SEP = 0.11%, RPD = 1.58; **Table 3.20**) as well as selected wavelengths (r^2 = 0.59, SEP = 0.11%, RPD = 1.57; **Table 3.24**).

Total soluble nitrogen

TSN predictions from dry land whole grain sample data obtained with the Büchi NIRFlex N-500 instrument (r^2 = 0.71, SEP = 0.06%, RPD = 1.84; **Table 3.11**) were acceptable for screening purposes in breeding programmes. Variable selection (**Table 3.12**) delivered slightly improved results; test set validation of selected variables delivered an r^2 of 0.73, SEP of 0.06% and RPD of 1.90, which could be used for screening purposes. Calibrations developed with the irrigation whole grain samples (**Table 3.11**) delivered a relatively acceptable model (r^2 = 0.50, SEP = 0.08%, RPD = 1.39). The r^2 value indicated the model was acceptable for rough screening, since more than 50% of the variance in the NIR data is accounted for by the variance in the reference data. Poor results were obtained with variable selection (r^2 = 0.47, SEP = 0.08%, RPD = 1.38, **Table 3.13**).

The Bruker MPA data (**Table 3.14**) delivered poor models for both dry land (r^2 = 0.44, SEP = 0.09%, RPD = 1.32) and irrigation whole grain samples (r^2 = 0.30, SEP = 0.10%, RPD = 1.20).

For the Büchi NIRLab N-200, whole grain dry land sample prediction delivered a r^2 of 0.55, SEP of 0.07% and RPD of 1.47 (**Table 3.15**), while variable selection also delivered relatively good results (r^2 = 0.53, SEP = 0.07%, RPD = 1.46, **Table 3.16**); both models were acceptable for rough screening. Very poor results were obtained for irrigation whole grain samples (r^2 = 0.03, SEP = 0.10%, RPD = 0.97; **Table 3.15**) and could not be used for future predictions, as also indicated by the very low RPD that refers to the poor sample range. No improvement was seen with variable selection (r^2 = 0.04, SEP = 0.10%, RPD = 1.01; **Table 3.17**).

The prediction of TSN from dry land flour samples with Büchi NIRLab N-200 data (**Table 3.15**) delivered acceptable results (r^2 = 0.59, SEP = 0.07%, RPD = 1.56) while a slightly better model

was obtained with variable selection (r^2 = 0.61, SEP = 0.07%, RPD = 1.59; **Table 3.18**). These models were acceptable for rough screening. Irrigation flour samples (**Table 3.15**) showed a slightly better prediction with an r^2 of 0.62, SEP of 0.06% and RPD of 1.61, and also delivered good calibrations with the process of variable selection (r^2 = 0.59, SEP = 0.06%, RPD = 1.54, **Table 3.19**); both these irrigation sample models were acceptable for rough screening.

The dry land whole grain models, developed with samples from the 2008 and 2009 harvest seasons (**Table 3.20**), proved to be acceptable for rough screening (r^2 = 0.59, SEP = 0.10%, RPD = 1.52). When test set validation was applied with selected variables (**Table 3.21**), results were unacceptable (r^2 = 0.33. SEP = 0.13%, RPD = 1.22). For irrigation whole grain samples (**Table 3.20**), a poor model was obtained (r^2 = 0.43, SEP = 0.09%, RPD = 1.23) and variable selection (r^2 = 0.50, SEP = 0.09%, RPD = 1.31; **Table 3.23**) only improved the results enough to be acceptable for rough screening purposes. Dry land flour samples (**Table 3.20**) delivered very poor results (r^2 = 0.37, SEP = 0.12%, RPD = 1.25) but the application of selected wavelengths (r^2 = 0.53, SEP = 0.11%, RPD = 1.44, **Table 3.22**) allowed the model to become acceptable for rough screening. Irrigation flour samples delivered poor results in both the case of the entire spectral region (r^2 = 0.40, SEP = 0.09%, RPD = 1.27, **Table 3.20**) and selected wavelengths (r^2 = 0.43, SEP = 0.09%, RPD = 1.26; **Table 3.24**).

Kolbach index

The prediction of KI from whole grain barley delivered poor results in most cases. For Büchi NIRFlex N-500 dry land whole grain samples (**Table 3.11**), a poor correlation (r^2 = 0.46, SEP = 4.07, RPD = 1.13) was found and variable selection (**Table 3.12**) delivered extremely poor results (r^2 = 0.11, SEP = 4.37 and RPD = 1.05). For irrigation whole grain samples (**Table 3.11**) an r^2 of 0.48 was obtained together with a SEP of 3.40 and RPD of 1.38, which was slightly better than for the dry land samples but still unacceptable for prediction purposes. Variable selection (**Table 3.13**) results proved acceptable for rough screening (r^2 = 0.59 SEP = 3.02, RPD = 1.50).

The Bruker MPA results (**Table 3.14**) for whole grain samples delivered unacceptable results for the prediction of KI from both dry land (r^2 = 0.09, SEP = 4.43, RPD = 1.05) and irrigation samples (r^2 = 0.27, SEP = 4.05, RPD = 1.16).

For the Büchi NIRLab N-200 data (**Table 3.15**), the correlation between predicted, from whole grain dry land barley, and reference values of KI was extremely poor (r^2 = 0.18, SEP = 3.11, RPD = 1.09) and the model developed with selected variables (**Table 3.16**) was unacceptable (r^2 = 0.10, SEP = 3.23, RPD = 1.05). Calibration development for irrigation whole grain samples (r^2 = 0.11, SEP = 3.32, RPD = 1.06; **Table 3.15**) was also unsuccessful and the use of selected variables (**Table 3.17**) caused no improvement (r^2 = 0.11, SEP = 3.32, RPD = 1.06).

The prediction of KI from Büchi NIRLab N-200 dry land flour spectra (r^2 = 0.20, SEP = 3.15, RPD = 1.08, **Table 3.15**) was also unsuccessful and variable selection (**Table 3.18**) delivered similar unacceptable results (r^2 = 0.20, SEP = 3.07, RPD = 1.11). Poor models were also obtained

for irrigation flour samples (r^2 = 0.39, SEP = 2.74, RPD = 1.28; **Table 3.15**) even with the application of variable selection (r^2 = 0.34, SEP = 2.81, RPD = 1.25; **Table 3.19**).

Free amino nitrogen

The calibration obtained from the Büchi NIRFlex N-500 dry land whole grain samples (r^2 = 0.77, SEP = 28.37 mg/L, RPD = 1.56; **Table 3.11**) proved to be acceptable for screening purposes. Variable selection delivered similar results (**Table 3.12**) to using the entire spectral region and was adequate for screening purposes since an r^2 of 0.68, SEP of 25.84 mg/L and RPD of 1.71 was obtained. In the case of irrigation whole grain samples (**Table 3.11**), a slightly poorer calibration, only acceptable for rough screening, was obtained (r^2 = 0.63, SEP = 29.05 mg/L, RPD = 1.54). Similar results were obtained with variable selection (r^2 = 0.63, SEP = 21.97 mg/L, RPD = 2.04; **Table 3.13**).

The Bruker MPA calibrations again delivered poor results (**Table 3.14**) that were generally unacceptable for future use (dry land whole grain: r^2 = 0.39, SEP = 34.80 mg/L, RPD = 1.28, and irrigation whole grain: r^2 = 0.38, SEP = 35.30 mg/L, RPD = 1.27).

The Büchi NIRLab N-200 whole grain dry land sample FAN calibration (r^2 = 0.36, SEP = 26.15 mg/L, RPD = 1.25; **Table 3.15**) delivered a poor correlation. The use of variable selection (**Table 3.16**) showed slightly better results (r^2 = 0.52, SEP = 21.93 mg/L, RPD = 1.49) that were appropriate for rough screening purposes. The model developed for irrigation whole grain samples (r^2 = 0.13, SEP = 30.87 mg/L, RPD = 1.07; **Table 3.15**) had a very poor relationship between predicted and actual reference values. Variable selection through uncertainty testing for irrigation whole grain samples (**Table 3.17**) also delivered disappointing results (r^2 = 0.22, SEP = 31.49 mg/L, RPD = 1.05).

Büchi NIRLab N-200 dry land flour data (**Table 3.15**) delivered good results (r^2 = 0.60, SEP = 21.02 mg/L, RPD = 1.56) that could be used for rough screening while similar results were obtained with variable selection (r^2 = 0.58, SEP = 21.23 mg/L, RPD = 1.54; **Table 3.18**). Irrigation flour sample results (**Table 3.15**) were also acceptable for rough screening (r^2 = 0.54, SEP = 22.86 mg/L, RPD = 1.45) whereas variable selection delivered even poorer results (r^2 = 0.49, SEP = 23.78 mg/L, RPD = 1.39; **Table 3.19**).

Diastatic power

DP predicted from the Büchi NIRFlex N-500 data (**Table 3.11**) for dry land whole grain samples delivered a good model (r^2 = 0.72, SEP = 59.42 W.K., RPD = 1.89) that was acceptable for screening purposes and comparable results were obtained with the use of variable selection (r^2 = 0.72, SEP = 59.70 W.K., RPD = 1.89; **Table 3.12**). Models developed for irrigation whole grain samples (**Table 3.11**) were very poor and could not be used for prediction purposes (R^2 = 0.40, SEP = 84.30 W.K., RPD = 1.27). The model remained very poor even with the use of only selected variables (r^2 = 0.40, SEP = 85.95 W.K., RPD = 1.24, **Table 3.13)**.

The Bruker MPA results (**Table 3.15**) for dry land whole grain samples were acceptable for rough screening purposes (r^2 = 0.59, SEP = 74.30 W.K., RPD = 1.53), while results for the irrigation samples (r^2 = 0.22, SEP = 94.70 W.K., RPD = 1.13) were extremely poor.

DP prediction from dry land whole grain sample Büchi NIRLab N-200 data (**Table 3.15**) proved to be acceptable for rough screening (r^2 = 0.56, SEP = 70.74 W.K., RPD = 1.48) and was successfully improved with the use of variable selection (r^2 = 0.73, SEP = 69.70 mg/L, RPD = 1.52, **Table 3.16**); the models could be used for screening purposes. Irrigation whole grain sample models (r^2 = 0.15, SEP = 71.90 mg/L, RPD = 1.06; **Table 3.15**) were unacceptable and did not show good results with variable selection either (r^2 = 0.15, SEP = 71.90 mg/L, RPD = 1.06; **Table 3.17**).

The Büchi NIRLab N-200 dry land flour sample model (**Table 3.15**) showed a poor correlation (r^2 = 0.47, SEP = 79.07 mg/L, RPD = 1.34) as did variable selection (r^2 = 0.49, SEP = 77.35 mg/L, RPD = 1.37, **Table 3.18**). The irrigation flour sample prediction model (r^2 = 0.58, SEP = 49.28 mg/L, RPD = 1.54, **Table 3.15**) was acceptable for rough screening purposes, but the use of uncertainty testing for variable selection did not deliver good results (r^2 = 0.44, SEP = 55.92 mg/L, RPD = 1.34).

Wort viscosity

The Büchi NIRFlex N-500 dry land whole grain data (**Table 3.11**) delivered very poor results (r^2 = 0.26, SEP = 0.02 cP, RPD = 1.38) and variable selection made no improvement (r^2 = 0.26, SEP = 0.02 cP and RPD = 1.38; **Table 3.12**). The model developed for irrigation whole grain samples (r^2 = 0.40, SEP = 0.02 cP, RPD = 1.67; **Table 3.11**) was also not acceptable for prediction purposes and variable selection (**Table 3.13**) again showed no improvement in the results (r^2 = 0.07, SEP = 0.03 cP and RPD = 1.23).

For the Bruker MPA dry land sample data (**Table 3.14**), an r^2 of 0.12, SEP of 0.02 cP and RPD of 1.07 were obtained. Results for irrigation samples (r^2 = 0.14, SEP = 0.03 cP and RPD = 1.08) were equally poor.

Prediction results from Büchi NIRLab N-200 data (**Table 3.15**) of dry land whole grain samples (r^2 = 0.34, SEP = 0.02 cP, RPD = 1.21) were very poor and variable selection (r^2 = 0.35, SEP = 0.02 cP, RPD = 1.22, **Table 3.16**) still delivered poor results. Irrigation whole grain samples (**Table 3.15**) delivered extremely poor correlations (r^2 = 0.25, SEP = 0.02 cP, RPD = 1.12) while the model developed with selected variables (**Table 3.17**) was also not usable (r^2 = 0.29, SEP = 0.02 cP, RPD = 1.08).

The prediction of viscosity from Büchi NIRLab N-200 dry land flour spectra (**Table 3.15**) delivered a poor correlation (r^2 = 0.43, SEP = 0.02 cP, RPD = 1.22), but the results obtained with variable selection (r^2 = 0.65, SEP = 0.02 cP, RPD = 1.30; **Table 3.18**) were acceptable for rough screening. The irrigation flour model (r^2 = 0.47, SEP = 0.02 cP, RPD = 1.37; **Table 3.15**) and

variable selection results (r^2 = 0.44, SEP = 0.02 cP, RPD = 1.32; **Table 3.19**) were also unacceptable.

Apparent attenuation limit

Prediction of AAL from Büchi NIRFlex N-500 dry land whole grain sample data (r^2 = 0.20, SEP = 1.73, RPD = 1.11; **Table 3.11**) delivered unacceptable results and the application of variable selection (**Table 3.12**) also resulted in a poor model (r^2 = 0.20, SEP = 1.72, RPD = 1.11). Irrigation sample prediction (r^2 = 0.22, SEP = 1.73, RPD = 1.10; **Table 3.11**) was also too poor to use, and again no improvement in the model was seen with the use of variable selection (r^2 = 0.22, SEP = 1.70, RPD = 1.12; **Table 3.13**).

Results for the Bruker MPA data (**Table 3.14**) of both dry land (r^2 = 0.17, SEP = 1.67, RPD = 1.09) and irrigation whole grain samples (r^2 = 0.05, SEP = 1.96, RPD = 0.97) were not usable for screening.

For the Büchi NIRLab N-200 data (**Table 3.15**), dry land whole grain samples delivered a very poor model (r^2 = 0.25, SEP = 1.60, RPD = 1.13) and variable selection (**Table 3.16**) did not improve these results (r^2 = 0.22, SEP = 1.65, RPD = 1.09); the models were too poor to be used for prediction purposes. Irrigation whole grain samples (**Table 3.15**) were also poorly predicted (r^2 = 0.20, SEP = 1.78, RPD = 1.07) and unacceptable even with selected variables (r^2 = 0.07, SEP = 1.85, RPD = 1.03; **Table 3.17**).

The models developed from Büchi NIRLab N-200 dry land flour samples, utilising the entire spectral region (r^2 = 0.25, SEP = 1.58, RPD = 1.14; **Table 3.15**) or only selected variables (r^2 = 0.26, SEP = 1.55, RPD = 1.16; **Table 3.18**), showed very poor correlations between predicted and actual AAL values. Irrigation flour sample results (r^2 = 0.23, SEP = 1.70, RPD = 1.12; **Table 3.15**) were unacceptable for screening purposes even with variable selection (r^2 = 0.18, SEP = 1.74, RPD = 1.10, **Table 3.19**).

Wort β-glucan content

A relatively poor correlation was obtained for the dry land whole grain sample Büchi NIRFlex N-500 data (r^2 = 0.46, SEP = 55.19 mg/L, RPD = 1.30; **Table 3.11**) and variable selection (**Table 3.12**) delivered extremely poor results (r^2 = 0.13, SEP = 24.75 mg/L, RPD = 2.90). Prediction of the β-glucan content from irrigation whole grain samples (**Table 3.11**) delivered a calibration acceptable for rough screening (r^2 = 0.61, SEP = 29.83 mg/L, RPD = 3.79) but the use of variable selection (**Table 3.13**) delivered disappointing results (r^2 = 0.36, SEP = 38.58 mg/L, RPD = 2.93).

Extremely poor models were obtained for dry land (r^2 = 0.07, SEP = 19.10 mg/L, RPD = 1.02) and irrigation (r^2 = 0.43, SEP = 37.50 mg/L, RPD = 1.27) whole grain Bruker MPA data (**Table 3.14**).

The Büchi NIRLab N-200 calibration results (**Table 3.15**) for the prediction of β-glucan content from whole grain dry land samples (r^2 = 0.29, SEP = 15.45 mg/L, RPD = 1.86) was very poor and

could not be used for prediction purposes. Variable selection (**Table 3.16**) was also carried out, but no improvement in the results were found (r^2 = 0.24, SEP = 16.35 mg/L, RPD = 1.76). The model for irrigation whole grain samples (r^2 = 0.23, SEP = 44.14 mg/L, RPD = 1.66; **Table 3.15**) was too poor for use, even after variable selection (r^2 = 0.30, SEP = 46.57 mg/L, RPD = 1.57; **Table 3.17**).

Poor correlations were also obtained from the Büchi NIRLab N-200 dry land flour data (r^2 = 0.25, SEP = 15.69 mg/L, RPD = 1.83; **Table 3.15**) and again variable selection (**Table 3.18**) showed no improvement (r^2 = 0.35, SEP = 14.64, RPD = 1.96). The irrigation flour based calibration model was poor (r^2 = 0.42, SEP = 38.39 mg/L, RPD = 1.90; **Table 3.15**) but more acceptable results were obtained with variable selection (**Table 3.19**), where the model (r^2 = 0.54, SEP = 33.83 mg/L, RPD = 2.16) was acceptable for rough screening purposes.

Discussion

Reference data

Most sample sets (**Figs. 3.1 - 3.6**) were characterized by the Gaussian or bell-shaped distribution of samples around the mean. Such a distribution will result in predictions at high values being lower than the true value, while those at the low end will appear higher than the actual reference results and, is likely to result in more accurate predictions for samples that are close to the mean. The Gaussian distributions observed for the reference data indicated samples were randomly selected and not specifically chosen for a certain known composition (Williams, 2001). The distributions of plumpness and β-glucan reference values were skewed (**Figs. 3.1 - 3.4b; Figs. 3.1- 3.4k**). Calibrations based on these sample sets predicted more accurately in the most populated reference value range. For example, high plumpness values were more accurately predicted than low plumpness values. Ideally, sample sets should be assembled with uniform distribution across the entire range (Williams, 2001).

NIR analysis (data analysis)

PCA analysis of the Bruker MPA data allowed the visualization of sample origin within the NIR spectral data set (**Fig. 3.8**). A clear difference was observed between samples cultivated under irrigation and dry land conditions. Samples grown under irrigation conditions tended to have higher and more consistent quality over localities due to the more controlled environment. Plumpness of the irrigation samples was mostly above 90%, indicating high starch content, whereas N values were in the ideal lower range of 1.7 – 1.9%. Dry land samples however, tended to have lower plumpness (70%) and higher N, and therefore, lower quality (F. Potgieter, SABBI, Caledon, South Africa, Personal Communication, 2009).

The two sample types (dry land and irrigation) were individually investigated. Of the nine dry land localities, four could be distinguished from the whole group, i.e. Swellendam, Tygerhoek, Klipdale and Caledon (**Fig. 3.9**). There was a clear tendency for samples cultivated at a specific locality to have similar spectral properties. In contrast, most of the samples from the irrigation

localities had similar spectral properties, which may have been due to the controlled irrigation conditions in all localities (**Fig. 3.10**). However, samples cultivated at Jan Kempdorp were distinguished from the remainder of the samples. This trial was removed from the breeding programme in 2008 because of its difference in quality. NIR spectroscopy in combination with PCA could allow for quick quality evaluation based on spectral data, which would allow barley breeders to save time and money on quality testing.

Moisture content

The three instrument types performed similarly with regard to the prediction of moisture content from whole grain barley (dry land r^2 0.47 – 0.53; irrigation r^2 0.08 – 0.19), and in all cases dry land samples were predicted more accurately than irrigation samples. Barley grown in irrigation regions tends to have higher quality than from in dry land areas, due to controlled irrigation. Therefore the irrigation samples had a larger percentage of samples that had higher moisture content than the dry land samples, as seen in **Figs. 3.1** and **3.2** (reference value distributions of dry land and irrigation samples). Variable selection did little to increase calibration accuracy and only substantially improved the Büchi NIRLab N-200 dry land sample calibration (r^2 increased from 0.47 to 0.60). The very low RPD values of these calibration models were an indication that the range of samples used was not adequate and required expansion; further research is therefore needed for moisture prediction from whole grain samples. The highest regression coefficient of the Büchi NIRLab N-200 calibrations was observed at 1940 nm, indicating that absorption at the wavelength associated with moisture contributed to the calibration model. NIR based moisture content determinations from whole grain barley are well established and the r^2 values obtained in this study did not compare well with those found in literature where values above 0.94 have been reported (Downey, 1985; Halsey, 1987; Sohn *et al.*, 2008). The comparatively small sample ranges utilised in this study [dry land samples = 8.20 - 12.59% (**Table 3.5**); irrigation samples = 6.95 – 10.81% (**Table 3.6**) compared to 9.64 - 18.45% used by Sohn et al. (2008)] account for the relatively poor calibrations.

Result obtained for moisture content prediction from ground barley delivered very good results (dry land r^2 = 0.76; irrigation r^2 = 0.69) but excellent results for the prediction of moisture content from ground barley have been reported in literature with r^2 values as high as 0.98 (Downey, 1985) and 0.99 (Henry, 1985). The reference value ranges [13.4 - 25.9% (Downey, 1985) and 5.9 – 16.8% (Henry, 1985)] used in these studies were much larger than the ranges used in this thesis (dry land = 8.20 – 12.59%; irrigation = 7.89 – 10.81%), which may account for the comparatively poor performance of South African barley calibrations. As seen with the whole grain samples, dry land flour predictions were more accurate than irrigation sample predictions and again this may be due to the smaller sample range used for irrigation samples (**Tables 3.7** and **3.8**). For the flour samples, the use of selected spectral variables (uncertainty testing) delivered similar results to the use of the entire spectral range. Calibrations based on flour samples may have been more

accurate than those of whole grain samples because the flour spectra were recorded immediately before the moisture determinations were performed, whereas the whole grain samples were scanned a few months later due to technical difficulties with the instrument. This may have resulted in moisture losses during the storage period.

Flour predictions were more accurate than whole grain predictions when samples from the two harvests seasons were combined. However, these calibrations were not more effective than those based on the 2008 harvest samples alone. Only the irrigation flour sample model developed with selected variables was good for screening. Both dry land (r^2 = 0.54) and irrigation (r^2 = 0.60) flour models were only adequate for rough screening purposes.

Plumpness

NIR has been evaluated by researchers to predict plumpness from whole grain barley and delivered good results: r^2 = 0.83, SEP = 11.5%, RPD = 2.4 (Edney *et al.*, 1994). The high SEP associated with this calibration was attributed to a poor range in percentage plumpness, where sample plumpness was skewed towards the high end of the range; which could also account for the low RPD (Edney *et al.*, 1994). However, this calibration was still acceptable for screening purposes (Williams, 2001). The range in reference values used (4.2% - 96.5%; Edney *et al.*, 1994) was much wider than the range used in this study [dry land samples = 78.0% – 99.1% (**Table 3.5**); irrigation samples = 63.7% – 99.6% (**Table 3.6**)], which may explain why poorer results were observed in this experiment. The reference value distributions (**Figs. 3.1** and **3.2**) of plumpness clearly indicated this property was skewed to the higher end of the range which also resulted in poor correlations. The three instruments delivered similar results for dry land samples (r^2 = 0.29 – 0.37) and the Bruker MPA and Büchi NIRLab N-200 delivered similar results for irrigation samples (r^2 = 0.49 – 0.52).

No reports on the prediction of plumpness from ground barley have been found in literature. Poor prediction results were not unexpected for this property; plumpness is a measure of kernel shape and size which could see a correlation to starch content. It was anticipated the NIR calibration for plumpness would rely on the differing scattering effects caused by different kernel shape or indirect correlation with starch content. The highest regression coefficients of the Büchi NIRLab N-200 calibrations were observed at 1900, 2000, 2252 and 2276 nm, indicating starch absorption contributed to the prediction of plumpness. In most cases, the results showed a poor correlation both for flour and whole grain, and apart from the irrigation whole grain (r^2 = 0.52 and 0.56) and flour calibrations (r^2 = 0.50), which would be acceptable for rough screening, none of the models were acceptable for prediction. Irrigation flour samples delivered slightly better results than dry land samples, due to the narrow range in reference values available for dry land samples (75.6 - 98.8%) when compared to irrigation samples (39.8 – 99.5%). Flour and whole grain calibrations had similar results for dry land (whole grain r^2 = 0.36; flour r^2 = 0.34) and irrigation (whole grain r^2 = 0.55; flour r^2 = 0.50) samples and variable selection did not improve any models remarkably. The

addition of samples from a second harvest season showed no improvement in either whole grain or flour results.

Extract

The Bruker MPA and Büchi NIRFlex N-500 data delivered very poor results for dry land whole grain samples (r^2 = 0.24 – 0.39) due to the narrow range (78.4% - 83.4%) in reference values. The range of samples needs to be expanded in order to obtain acceptable calibrations. Good models were developed for irrigation samples and could be used for rough screening purposes. The use of spectral variable selection only improved the Büchi NIRFlex N-500 irrigation whole grain sample model, where the r^2 was improved from 0.60 to 0.69 and would be acceptable for screening purposes.

The results obtained from the three instruments did not compare well with data reported in literature. Researchers have developed calibrations adequate for screening (Williams, 2001) extract potential ($r^2 \geq 0.78$) of whole grain barley (Halsey, 1987; Black & Panozzo, 2001); the result in this study was inadequate in comparison to these previous studies. The wider range in reference values (77 - 87%) used by Black & Panozzo (2001) contributed to more accurate predictions. Successful models have also been developed for the prediction of extract from whole grain malt (Black & Panozzo, 2001) where an r^2 of 0.76 and SEP of 1.00% was obtained. A very good model was developed for the prediction of extract from wort: r^2 = 0.88 and SEP = 0.9% (Ratcliffe & Panozzo, 1999). It has been stated that an accurate calibration developed for whole grain malt would not be suitable for barley; malting changes the structure of grain such that malt contains more loosely bound protein and is softer than barley, allowing NIR radiation to penetrate more deeply into malt than barley (Halsey, 1987). The activity of the enzymes during malting also influences the malt extract and limits the accuracy of any NIR prediction based on unmalted barley (Henry, 1985).

The models developed for flour showed no improvement over the whole grain models, but the calibration developed for the irrigation flour samples (r^2 = 0.55) could be used for rough screening purposes. A poor model for the prediction of extract from ground barley has been reported (r^2 = 0.49, SEP = 1.65% (Morgan & Gothard, 1979)), it was reasoned the NIR method could not account for enzyme activity and subsequent changes that occur in the grain during malting. These researchers added that expansion of the sample range is important for the development of accurate calibration models (Morgan & Gothard, 1979). A number of researchers showed NIR spectroscopy can be used to predict extract from ground barley, obtaining r^2 values as high as 0.96 (McGuire, 1982), 0.88 (Henry, 1985) and 0.77 (Tragoonrung *et al.*, 1990); all of which would be acceptable for screening purposes. The wider sample ranges [46.9 – 62.2% (Henry, 1985) and 71.3 – 85.1% (Tragoonrung *et al.*, 1990)] applied in these studies, when compared to those used in this thesis (dry land range: 78.4 – 83.4%; irrigation range: 77.7 – 83.6%), contributed to more accurate predictions. Variable selection did not improve any of the flour calibrations and the

addition of samples from the 2009 harvest season did not improve any of the results for extract prediction.

Total nitrogen

In the case of both the Büchi NIRFlex N-500 and Bruker MPA data, dry land and irrigation whole grain samples delivered similar results. For the Büchi NIRLab N-200, whole grain dry land predictions (r^2 = 0.79) were more accurate than irrigation sample prediction (r^2 = 0.27) due to the wider sample range obtained for dry land samples (1 – 2.05%) compared to irrigation samples (1.28 – 2.08%). Models using selected variables showed no improvement on models using the entire spectrum, except for the irrigation whole grain sample model obtained with the Büchi NIRFlex N-500 (r^2 increased from 0.78 to 0.85, and would therefore be acceptable for use in most applications). The prediction of nitrogen content from whole grain barley is well established in literature and good calibrations for this property are expected. The results found in this study compared well with that of previous reports (Halsey, 1987; Li *et al.*, 1995), although some were able to develop excellent calibration models for whole grain barley with r^2 = 0.94 (Edney *et al.*, 1994) and 0.95 (Sohn *et al.*, 2008). The wider samples ranges used by these researchers [9.4-15.5 (Edney *et al.*, 1994) and 6.81 – 12.22 (Sohn *et al.*, 2008)] contributed to their good results.

More effective models were obtained for dry land flour samples (r^2 = 0.84) than for irrigation flour samples (r^2 = 0.65). These results were inferior to previous reports in literature. Values of r^2 as high as 0.99 (Henry, 1985) and 0.92 (Gill *et al.*, 1979; Tragoonrung *et al.*, 1990) have been reported; both these studies used selected wavelengths for calibration development. In this study variable selection did not increase r^2 values for this property. The highest regression coefficients of the Büchi NIRLab N-200 calibrations, observed at 1980 and 2050 nm, corresponded with protein absorptions and indicate protein contributed to total nitrogen content calibrations.

Although most of the combined harvest season results were acceptable for rough screening purposes no improvement on the results obtained with the 2008 harvest season alone was made. The irrigation whole grain model did however improve from r^2 of 0.27 to 0.37, and r^2 of 0.10 to 0.49 with variable selection, with the addition of a second harvest season.

Total soluble nitrogen

Dry land samples were predicted with more accuracy than irrigation samples for all three instruments, even though the sample ranges were similar for dry land and irrigation samples. The reference value distribution for the irrigation samples (**Fig. 3.2**) was skewed towards the lower end of the range, while the dry land samples showed more samples centred around the mean, which would have influenced calibration accuracy. The models developed from flour (dry land r^2 = 0.59; irrigation r^2 = 0.62) were better than those obtained with whole grain samples (dry land r^2 = 0.55; irrigation r^2 = 0.03) for the Büchi NIRLab N-200. The use of uncertainty testing for variable selection did not substantially improve the calibration models. The Büchi NIRFlex N-500 predicted

dry land whole grain samples with greater accuracy (r^2 = 0.71) than the other two instruments, while the Büchi NIRLab N-200 showed a very poor prediction for irrigation whole grain samples (r^2 = 0.03). The highest regression coefficients for Büchi NIRLab N-200 data were observed around 1980 nm, which corresponds with protein and indicated protein content contributed to the calibrations.

These results showed an improvement over previous reports, where very poor results (r^2 = 0.01) were found when TSN was predicted from whole grain barley (Black & Panozzo, 2001). This previous poor performance was attributed to the complex nature of this constituent within unmalted barley; therefore researchers were only able to develop a relatively acceptable model for the prediction of TSN from whole grain malt (r^2 = 0.53, SEP =0.3%) (Black & Panozzo, 2001).

The addition of a second harvest season did not improve the results significantly, with the exception of the model for irrigation whole grain samples for which the r^2 value went from 0.04 to 0.50 and became acceptable for rough screening purposes.

Kolbach index

Models developed from the Büchi NIRFlex N-500 whole grain data (r^2 of 0.46 for dry land samples and 0.48 for irrigation samples) were better than the results obtained from the other two instruments. Uncertainty testing of the irrigation whole grain samples scanned with the Büchi NIRFlex N-500 proved to be acceptable for rough screening purposes (r^2 = 0.59). Even though flour samples delivered slightly better results than whole grain, the correlations were poor and could not be used in future predictions. The addition of samples from a second harvest season did not improve any of these results. If relatively accurate and acceptable calibrations are however developed for TN and TSN, the KI can be calculated from these predicted results and would deliver similarly accurate results.

Free amino nitrogen

The Büchi NIRFlex N-500 whole grain data delivered better results than the other two instruments, and dry land (r^2 = 0.77) and irrigation (r^2 = 0.63) models developed from the N-500 data were acceptable for at least rough screening purposes. Uncertainty testing was only successful for the Büchi NIRLab N-200 dry land whole grain samples, where the r^2 increased from 0.36 to 0.52, and was thus be acceptable for application as a rough screening method. In all cases, dry land samples were predicted with better accuracy than irrigation samples, probably because of the wider sample range available for dry land samples (107 – 286 mg/L) when compared to irrigation samples (99 – 252 mg/L). Literature reported even poorer results for the prediction of FAN from whole grain barley with r^2 values as low as 0.10 and a SEP of 31 mg/L (Black & Panozzo, 2001), therefore the Büchi NIRFlex N-500 results are an improvement on previous work. Smaller sampler ranges (92 - 228 mg/L) (Black & Panozzo, 2001) used by these researchers contributed to these poor results. In previous reports, acceptable results were only obtained with the prediction of FAN

from whole grain malt (r^2 = 0.63, SEP = 17 mg/L) (Black & Panozzo, 2001) and wort (r^2 = 0.73, SEP = 15 mg/L) (Ratcliffe & Panozzo, 1999).

For dry land and irrigation samples, flour delivered more accurate predictions than whole grain, and dry land samples were predicted better than irrigation samples. No results for the prediction of FAN from ground barley have been reported in literature. Uncertainty testing did not improve the flour calibrations at all and the addition of samples from a second harvest season did not improve any of the calibration results.

Diastatic power

The Bruker MPA and Büchi NIRLab N-200 instruments delivered similar results for dry land and irrigation whole grain samples, while the Büchi NIRFlex N-500 instrument generated more accurate predictions for both sample types. Uncertainty testing only improved the Büchi NIRLab N-200 whole grain model (r^2 value increased from 0.56 to 0.73), making it acceptable for screening purposes. All three instruments showed that dry land samples were better predicted than irrigation samples and this could be attributed to the smaller range of irrigation reference values (170 W.K. – 554 W.K. versus 170 W.K. – 635 W.K. for the dry land samples). Acceptable calibrations (R^2 = 0.59) for predicting DP with cross-validation from whole grain barley were reported in literature (Li *et al*., 1995). A very poor calibration was also reported (r^2 = 0.39, SEP = 57 W.K.) which was due to the relatively small range in reference values used by these researchers (174 – 549 W.K.) (Black & Panozzo, 2001). Poor results can also be attributed to the inability of the NIR method to account for the complex interactions of barley endosperm substrates and enzymes during malting and the extent of endosperm modification as a result thereof (Henry, 1985a; Li *et al*., 1995). More acceptable results were therefore obtained for the prediction of DP from whole grain malt (r^2 = 0.54, SEP = 54 W.K.) (Black & Panozzo, 2001).

The model developed from dry land flour samples (r^2 = 0.47) with the Büchi NIRLab N-200 was not as effective as the whole grain model (r^2 = 0.56), while the irrigation sample flour model (r^2 = 0.58) delivered better results than the whole grain model (r^2 = 0.15). Variable selection did not improve any of the flour calibrations. Predictions for DP from ground barley have not been reported in literature and the addition of a second harvest season did not improve the calibration results.

Wort viscosity

The three instruments performed similarly in the prediction of wort viscosity from whole grain barley and results were generally unacceptable for use. Irrigation sample wort viscosity was predicted less accurately than dry land sample wort viscosity, even though the range in reference values for the two sample types were very similar (**Tables 3.5** – **3.8**). Uncertainty testing did little to improve on the whole grain models developed with the entire spectral region. Variable selection improved only the dry land flour model and with an r^2 increased from 0.43 to 0.65 it was acceptable for screening purposes. Flour samples showed more accurate predictions than whole grain samples.

The addition of a second harvest season showed no improvement on these results. These models did not compare well with reports in literature, where acceptable cross-validation models were obtained (R^2 = 0.62, SEP = 0.02 cP) for wort viscosity prediction from whole grain unmalted barley (Li *et al.*, 1995) and ground barley (r^2 = 0.65, SEP = 0.60 cP) (Allison *et al.*, 1978). The bulking of sample replicates contributed to these poor results, since the reference values were not representative of the specific sample spectra used for calibration development. Further research is needed to develop suitable prediction models for this property.

Apparent attenuation limit

All three instruments delivered similar results for dry land and irrigation samples, and both whole grain and flour sample predictions were similarly poor. All calibrations for the prediction of AAL from whole grain and ground unmalted barley are unacceptable for use in NIR prediction of this property. The addition of a second harvest season did not improve the sample range and showed no improvement in these results. These poor correlations for dry land and irrigation samples can only be attributed to the fact the NIR spectra of unmalted barley cannot account for the action of yeast on fermentable sugars during fermentation and can therefore not predict AAL from unmalted barley. No results have been reported in literature for the prediction of AAL from either whole grain or ground unmalted barley.

Wort β-glucan content

The results obtained from all three instruments were very poor, except for the Büchi NIRFlex N-500 model that was developed for irrigation whole grain samples (r^2 = 0.61). Irrigation samples mostly predicted more accurately than dry land samples. This was due to the smaller range in wort β-glucan content for dry land samples (49 – 342 mg/L) compared to irrigation samples (35 – 439 mg/L). **Figs 3.1** and **3.2** showed that reference values were skewed to the lower end of the range which also influenced prediction accuracy. Variable selection did little to improve the results. Unacceptable calibrations for the prediction of β-glucan content from whole grain barley have been reported in literature, where an r^2 of 0.25 and SEP of 240 mg/L was obtained (Black & Panozzo, 2001). This low calibration accuracy may have been due to the complex nature of the β-glucans, and proteins and starches which are not yet modified by the action of enzymes during malting (Black & Panozzo, 2001). Despite this however, Sohn *et al.* (2008) successfully predicted β-glucan content from whole grain barley (r^2 = 0.80, RMSEP = 0.43 mg/L); the model was suitable for screening purposes and could be used for classification of barley into low and high groups (Sohn *et al.*, 2008). The β-glucan content was also successfully predicted from whole grain malt (r^2 = 0.51, SEP = 165 mg/L) but only accurately enough for rough screening purposes (Black & Panozzo, 2001).

Although flour samples predicted better than whole grain samples, the results were still very poor. In most cases irrigation samples were predicted more accurately than dry land samples.

Uncertainty testing only improved the irrigation flour model r^2 value from 0.42 to 0.54, which was acceptable for screening. Three previous studies predicted β-glucan content from ground barley with r^2 values of 0.76 (Allison *et al.*, 1978) and 0.77 (Henry, 1985; Szczodrak *et al.*, 1992). The addition of a second harvest season showed absolutely no improvement over results obtained in this study. The poor distribution of reference values in the sample range may be the reason for the poor results that were obtained. Viscosity is a measure of the breakdown of β-glucans during malting (Kotze, 2009) and both these properties were predicted poorly in this study. Therefore further research is needed to develop suitable prediction models for the prediction of wort β-glucan content.

Conclusion

The application of PCA to spectral data made it possible to distinguish between irrigation and dry land samples, as well as between samples cultivated at specific localities. It was not possible to distinguish between samples from all dry land and irrigation localities, due to the similarity in environment (and therefore spectral properties) of the respective dry land and irrigation localities. The low RPD values of most calibrations are an indication that the range of samples used in calibration development was not adequate and must be expanded for future work. Since these calibrations will only be used for rough screening purposes, i.e. for the separation of potentially good quality cultivars from very poor malting cultivars, and will not be used for quality control, the low RPD values do not pose a problem at this stage. The differences seen between calibrations from the three instruments may be due to sample presentation and the sample cells that were used for the different instruments. The most important aspect to be kept in mind was that sample replicates were bulked before micro-malting. The three samples were however scanned separately, resulting in three spectra with an averaged malt quality reference value for all malt properties. The reference values of the respective malt properties, as determined with micro-malting, were therefore not representative of the specific sample spectra that were recorded, which had a large impact on the calibration models and subsequent predictions. More accurate prediction models can only be attained if each sample is malted individually so that reference values obtained through micro-malting will be representative of that specific sample. Although NIR prediction of malt properties from whole grain barley cannot account for enzyme action during malting or the action of yeast during brewing, the technique shows potential to be used as a rough screening method in earlier generations, which would allow for the elimination of poor malting cultivars early on in the breeding programme. The standard error of laboratory (SEL) for the micro-malting technique could not be obtained in this study and therefore there is no knowledge on the precision of the reference methods compared to that of the NIR method. Poor calibration results could be due to poor precision of the reference method used. Relatively average models with high error values could be implemented for rough screening; if the error of the model is known breeders could compensate for this error and use the model for rough estimations of properties. Given these

models will not be used for quality control but only for rough screening, they generally prove acceptable for quick evaluation of the thousands of breeding lines that need to be tested in the earlier stages of the breeding programme.

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Chapter 4

Use of principal component analysis (PCA) biplots to study the genotype-by-environment (GxE) interaction of malting barley in a South African breeding programme

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Use of principal component analysis (PCA) biplots to study the genotype-by-environment (GxE) interaction of malting barley in a South African breeding programme

Abstract

The first two principal components of principal component analysis (PCA) biplots were evaluated to illustrate the relationship between genotypes and environments of nine malt properties (extract, total nitrogen (TN), total soluble nitrogen (TSN), Kolbach Index (KI), free amino nitrogen (FAN), diastatic power (DP), wort viscosity, apparent attenuation limit (AAL) and wort β-glucan content) for dry land and irrigation areas over the 2008 and 2009 growing seasons. Several lines were consistent with respect to the investigated properties over both seasons and locations. However, seasonal differences were also evident and the genotype-by-environment (GxE) interaction should be studied over an additional season to confirm the consistency in quality over seasons. This study illustrates and confirms the efficient use of PCA biplots to select lines which possess acceptable malting quality characteristics over multiple seasons to progress to the next stage in the breeding programme. Similarly, PCA biplots can be used to identify localities that provide the optimal conditions for specific lines or are ideal for the production of desired characteristics. The technique is an additional tool to visually assess the influence of breeding lines (cultivars) and localities simultaneously. More accurate results could be achieved with analysis of variance (ANOVA) but this method could not be applied, since every genotype must occur at every site, and an imbalance in genotypes by locations was present in this study.

Introduction

Malting barley is a highly specialised cereal and has a long breeding and malting tradition. The quality specifications for malting barley are the most challenging specifications in comparison to other cereals (Kreisz, 2009). Malting barley commands a premium price over feed barley; since malting barley can be designated as feed grade if it does not possess acceptable malting quality characteristics (Ullrich, 2002) farmers wish to know which cultivars are best for their environment (Kotze, 2009).

Initially, breeding programmes are concerned with early stages of quality evaluation where large numbers of new breeding lines are grown in a small number of field trials. The best lines are selected to continue to the next stage of testing, which results in fewer lines being evaluated in more locations. The process culminates in the testing of a small number of elite breeding lines in a large number of trials that span a wide range of geographic locations and several growing seasons. On the basis of these trials new breeding lines can be recommended for commercial use and make the transition to commercial cultivar. The main objective of these trials is to identify the best lines for cultivation and use (Smith *et al.*, 2001).

The recommendation of new lines for commercial use requires reliable and accurate characterisation for each line across a range of target environments. This requires the assessment of overall performance of each line (across all environments) and whether performance is affected by the environment or there is a genotype-by-environment (GxE) interaction (Smith *et al*., 2001). Variation in barley quality from year to year has been identified as a major problem in the brewing industry and is mostly attributed to differences in genotype and environmental conditions (Savin & Molina-Cano, 2002); GxE therefore has important implications in breeding programmes (Voltas *et al*, 2002).

Because South African malting barley is cultivated in two regions, i.e. dry land (Southern Cape) and irrigation (Northern Cape), different genotypic expression across environments complicate the selection of cultivars with superior quality from these two regions. Plant breeders conduct large scale trials to investigate the performance of large numbers of genotypes in several environments that represent the target area for the breeding programme (Voltas *et al*, 2002). The aim is to select the best genotypes for the purpose of further crop improvement. The data from such trials usually consist of a number of attributes for each genotype in each environment. There is a need to analyse the two-way GxE interaction in such a way so systematic patterns can be assessed and their relevance evaluated (Kroonenberg, 1995).

Principal component analysis (PCA) biplots which show both genotypes and environments simultaneously (Gabriel, 1971), allow for the display of those dimensions which account for the maximum amount of variation. Genotype markers are represented by points and environment markers by vectors (Kempton, 1984; Kroonenberg, 1995) and, subsequently, the obtained response of a line over different environments may be visualised. Interactions between genotype and environment vectors are positive for acute angles (less than 90°), negative for obtuse angles (90-180°) and negligible for right angles (90°) (Chapter 2.10) (Kroonenberg, 1995; Voltas *et al*., 2002). These biplots can allow plant breeders to select potential good malting cultivars from the breeding programmes, with regard to the response of certain properties to certain environments over subsequent growing seasons. In this study the use of PCA biplots was evaluated as an additional tool to visually assess the influence of breeding lines (cultivars) and localities simultaneously.

Materials and Methods

Samples and reference data

Barley samples were obtained from the South African Barley Breeding Institute (SABBI) 2008 and 2009 breeding trials and were grown either under irrigation (2008: 216 samples, 12 lines, 5 localities; 2009: 215 samples, 8 lines, 5 localities) or under dry land conditions (2008: 312 samples, 15 lines, 7 localities; 2009: 224 samples, 9 lines, 6 localities). Dry land localities included Napier, Klipdale, Bredasdorp, Caledon, Swellendam and Heidelberg, while irrigation localities included Luckhoff, Douglas, Rietrivier, Hartswater and Taung. All lines are summarised in **Table 4.1**. Dry

land samples were laid out as a rectangular array of plots with 15 rows by 5 columns, where each block of 5 rows constituted a complete replicate (75 samples per locality). Irrigation samples were laid out as a rectangular array of plots with 12 rows by 4 columns, where 4 rows constituted a complete replicate (48 samples per locality). Replicates from field trials were bulked and the samples were malted on a small scale as mentioned in Chapter 3.

Table 4.1 Lines obtained from dry land and irrigation localities in 2008 and 2009

Dry land		**Irrigation**	
2008	**2009**	**2008**	**2009**
M1	M1	M3	M16
M2	M2	M16	M17
M3	M3	M17	M18
M4	M4	M18	M19
M5	M6	M19	M21
M6	M7	M20	M22
M7	M8	M21	M23
M8	M10	M22	M25
M9	M13	M23	
M10		M24	
M11		M25	
M12		M26	
M13			
M14			
M15			

Data analysis

Values for a single reference property were arranged in a matrix where rows were cultivars and columns were localities (**Appendix 2**). PCA was applied to the reference data (extract, total nitrogen (TN), total soluble nitrogen (TSN), Kolbach Index (KI), free amino nitrogen (FAN), diastatic power (DP), wort viscosity, apparent attenuation limit (AAL), β-glucan content) for the dry land and irrigation samples over both seasons (2008 and 2009) individually. The GxE relationship was displayed by means of biplots showing PCA scores and loadings for the first two principal components. XLSTAT (version 2010.5.05, Addinsoft, Paris, France) was used for PCA and to display biplots followed by calculation of the correlation matrix for all malt properties based on growing conditions (dry land and irrigation) and harvest season (2008 and 2009).

Results

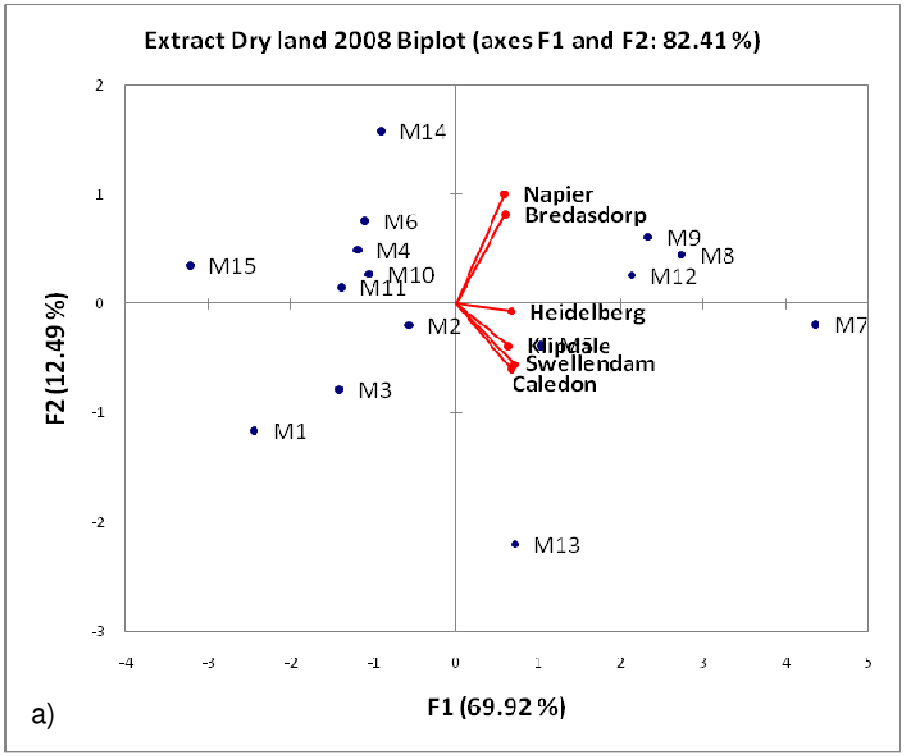
PCA biplots for the nine malt properties from the dry land and irrigation areas are illustrated in **Figs. 4.1** to **4.18**. Correlation matrices for malting data of dry land and irrigation samples (2008 and 2009) are summarized in **Tables 4.2** to **4.5**.

Extract

PCA biplots for the dry land samples (**Fig. 4.1**) illustrated that M7, M8 and M13 had high extract, and M1, M3 and M10 had consistently low extract over both seasons. Some variation was also seen over seasons, where M2 had low extract for Napier and Bredasdorp in 2008 but high extract for these sites in 2009. M6 had very high extract for Napier and Bredasdorp in 2008, whereas in 2009 it had low extract for these two sites. M4 showed low extract values in 2008 for Heidelberg, Klipdale, Swellendam and Caledon but high values for these localities in 2009. M15 and M11 had very low extract values for most sites in 2008 and were subsequently removed from the 2009 trials. M2 and M8 had very high extract for Napier in 2009, while M4 had higher extract in Klipdale for this season.

The PCA biplots for the irrigation samples (**Fig. 4.2**) showed that M16, M17, M18, M19, M21, M22 and M25 had high extract for most localities over both seasons. Some variation over seasons occurred where M23 had high extract in 2008 but low extract for the 2009 season, especially for Hartswater. Lines such as M3, M24 and M26 had very low extract in the 2008 harvest, and were excluded in 2009. M18 had consistently high extract in Rietrivier, whereas M23 had low extract for this location over both seasons.

For dry land sample property correlations (**Table 4.2**), extract was negatively correlated ($P < 0.01$) with TN, TSN, FAN and wort viscosity and positively correlated with KI ($P < 0.05$) and AAL ($P < 0.01$) in the 2008 season. According to **Table 4.4,** extract was negatively correlated ($P < 0.01$) with TN, TSN, FAN and DP and positively correlated ($P < 0.01$) with KI, AAL and wort viscosity in the 2009 season. Irrigation sample properties (**Tables 4.3** and **4.5**) showed consistent results over the two seasons; extract was negatively correlated ($P < 0.01$) with TN, TSN, FAN, DP, wort viscosity and AAL.



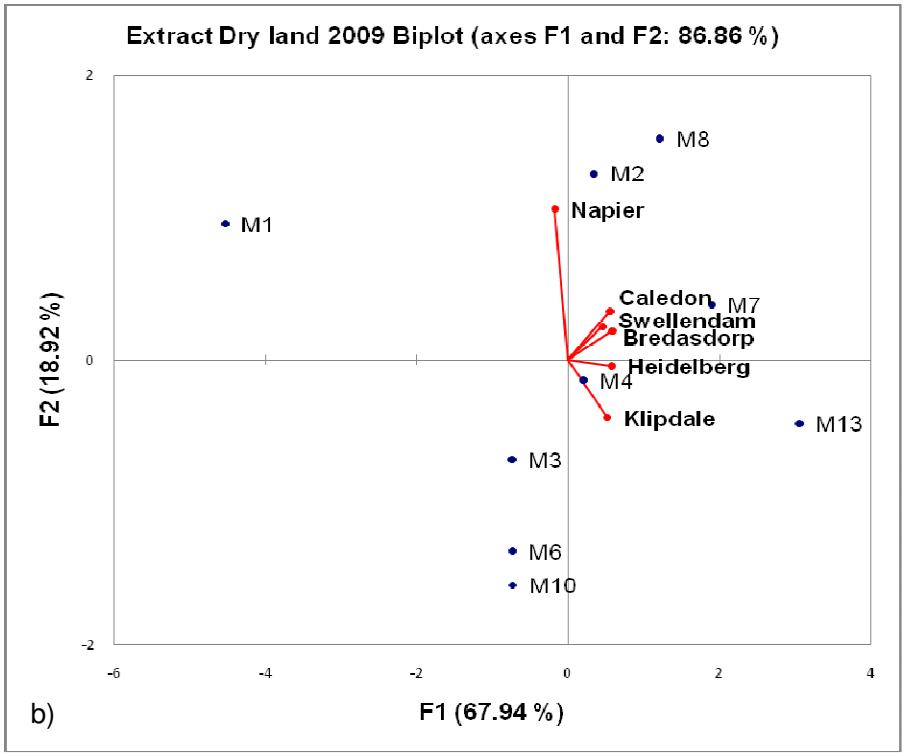


Figure 4.1 PCA biplots for extract of dry land samples from a) 2008 and b) 2009.

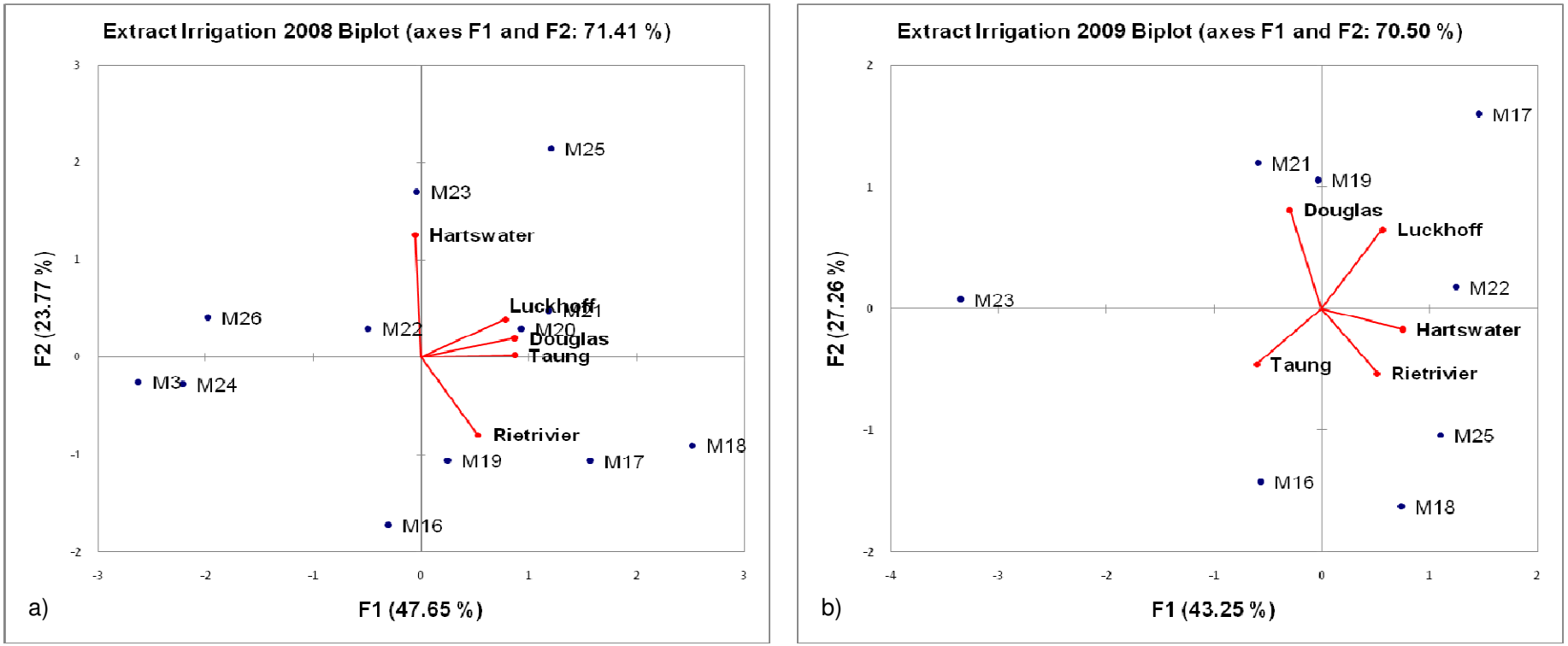


Figure 4.2 PCA biplots for extract of irrigation samples from a) 2008 and b) 2009.

Total Nitrogen (TN)

Most dry land lines had high TN content in 2008, with M2 the highest (**Fig. 4.3**). M7 was the only line with low TN for most locations over both seasons. Seasonal differences were observed, where M6 and M10 had low TN for sites such as Swellendam, Klipdale and Napier in 2008 but high TN in 2009, whereas M4 had high TN for these sites in 2008 and low TN in 2009. M8 and M3 had consistently higher TN for Heidelberg over both years, and M1 for Caledon. M14 and M15 had high TN for most locations in 2008 and were consequently omitted from the 2009 trials.

Fig. 4.4 indicated that M21 was the only line with low TN values over both seasons for all irrigation localities. M16 had high TN for all localities, whereas M17, M19 and M22 had high TN for Hartswater and Douglas in 2008 and most localities in 2009. M24 had very high TN in 2008 and was removed from the breeding programme in 2009. The biplots also showed that seasonal differences occurred. For example, M23 had high TN values in 2008 for Hartswater and Douglas, but low TN for these localities in 2009. M18 had low TN in 2008 but high TN in 2009 for Rietrivier, Luckhoff and Taung.

Dry land TN (**Table 4.2**) showed a negative correlation ($P < 0.01$) with extract and AAL but positive correlations ($P < 0.01$) with TSN, FAN and DP in the 2008 season. In 2009 (**Table 4.4**), TN was negatively correlated ($P < 0.01$) with extract, KI, wort viscosity and AAL, but positively correlated ($P < 0.01$) with TSN, FAN, DP and wort β-glucan content. TN for the 2008 irrigation areas was negatively correlated ($P < 0.01$) with extract and positively correlated ($P < 0.01$) with TSN, FAN, wort viscosity and wort β-glucan content (**Table 4.3**) whereas in 2009, TN was negatively correlated ($P < 0.01$) with extract, KI and AAL, but positively correlated ($P < 0.01$) with TSN, FAN, DP, wort viscosity and β-glucan content.

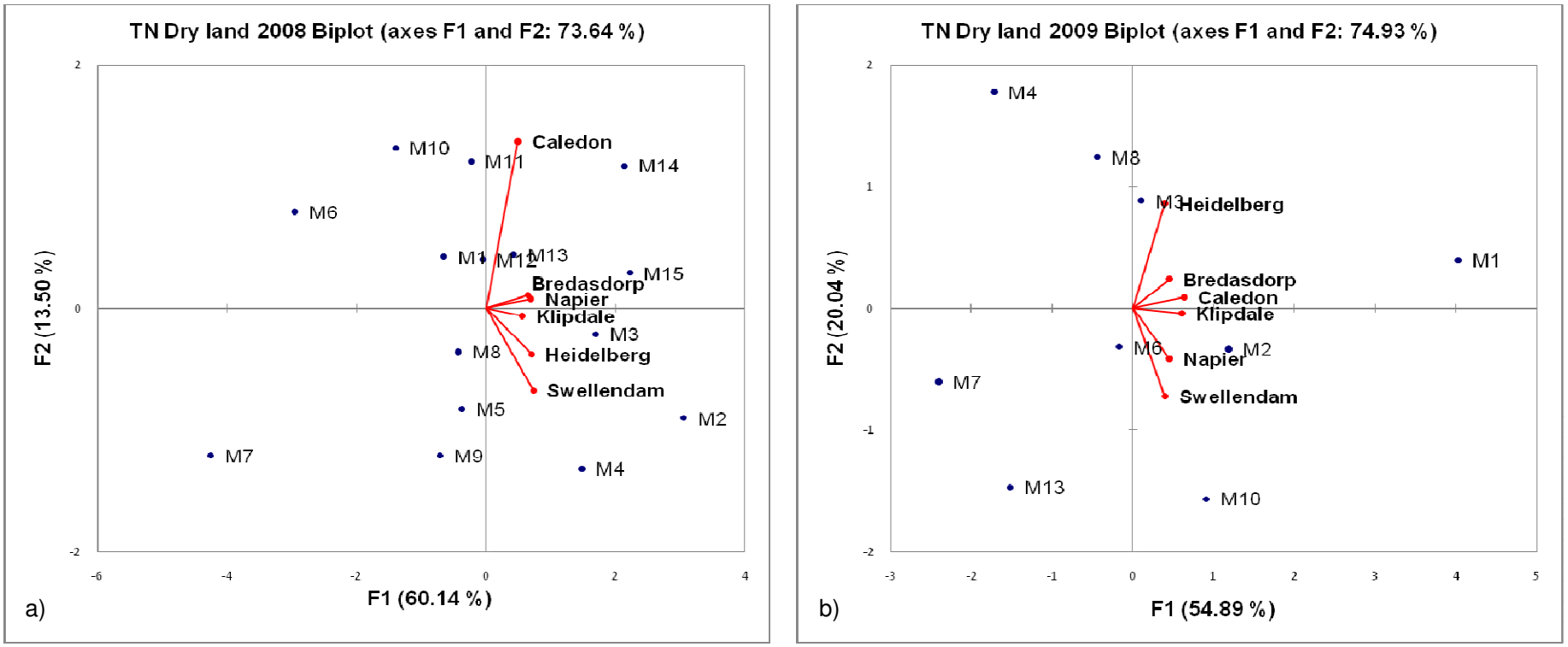


Figure 4.3 PCA biplots for total nitrogen (TN) of dry land samples from a) 2008 and b) 2009.

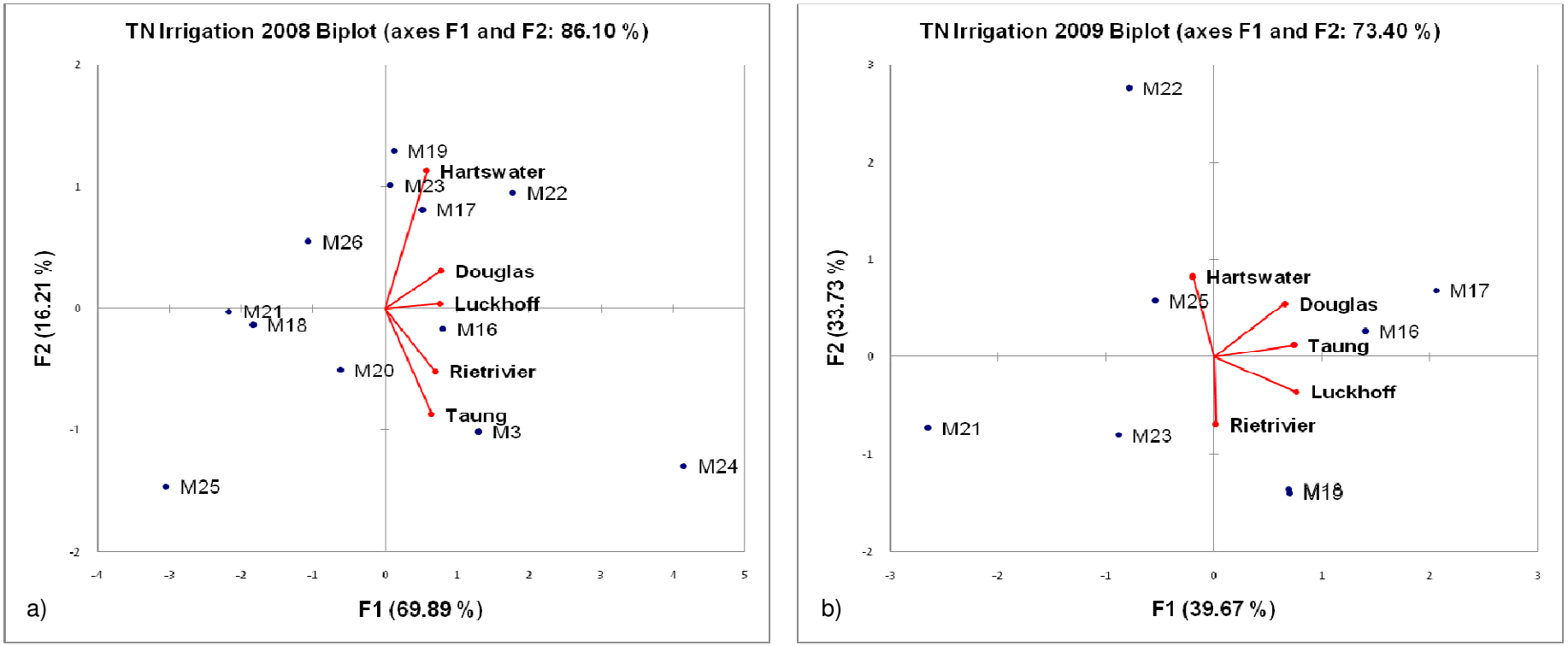


Figure 4.4 PCA biplots for total nitrogen (TN) of irrigation samples from a) 2008 and b) 2009.

Total Soluble Nitrogen (TSN)

Fig. 4.5 showed that M2, M3, M4, M8 and M10 had high TSN in both seasons for the dry land areas. M2 had consistently high TSN values for Napier over both years, whereas M4 had high TSN for Heidelberg over both years. M6, M7 and M13 had low TSN for most localities for the 2008 and 2009 season. Seasonal differences were also observed, where M1 had low TSN in 2008 and high TSN in 2009. M14 had high TSN in 2008 and was not included in 2009.

PCA biplots for irrigation samples (**Fig. 4.6**) indicated that M23 had the highest TSN for most locations in 2008 as well as 2009. M18, M19, M21 and M25 had low TSN for most localities, whereas M16 and M17 had high TSN over both years. Seasonal variation was seen, where M21 had higher TSN for Rietrivier in 2008 but low TSN for this locality in 2009. M23 had high TSN especially for Rietrivier in both seasons.

TSN for the dry land 2008 season (**Table 4.2**) showed significant negative correlations ($P < 0.01$) with extract, wort viscosity, wort β-glucan content and AAL ($P < 0.05$) and significant positive correlations ($P < 0.01$) with TN, KI, FAN and DP. In 2009 (**Table 4.4**), TSN was negatively correlated ($P < 0.01$) with extract, wort viscosity and β-glucan content and positively correlated ($P < 0.01$) with TN, FAN and DP. In 2008, TSN for the irrigation samples showed a negative correlation ($P < 0.01$) with extract and significant positive correlations ($P < 0.01$) with TN, KI, FAN and wort viscosity (**Table 4.3**). In 2009, however, this property was negatively correlated ($P < 0.01$) with extract and AAL and positively correlated ($P < 0.01$) with TN, KI, FAN, DP and wort viscosity (**Table 4.5**).

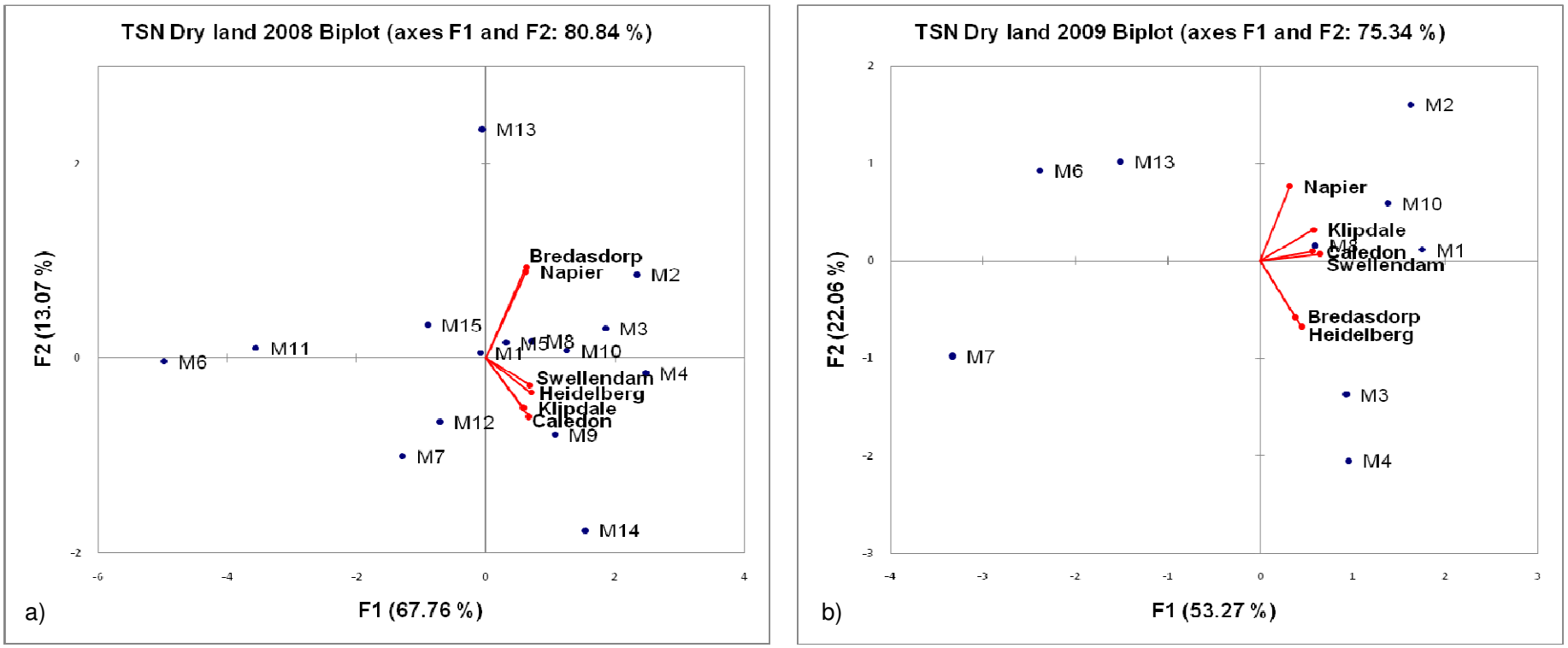


Figure 4.5 PCA biplots for total soluble nitrogen (TSN) of dry land samples from a) 2008 and b) 2009.

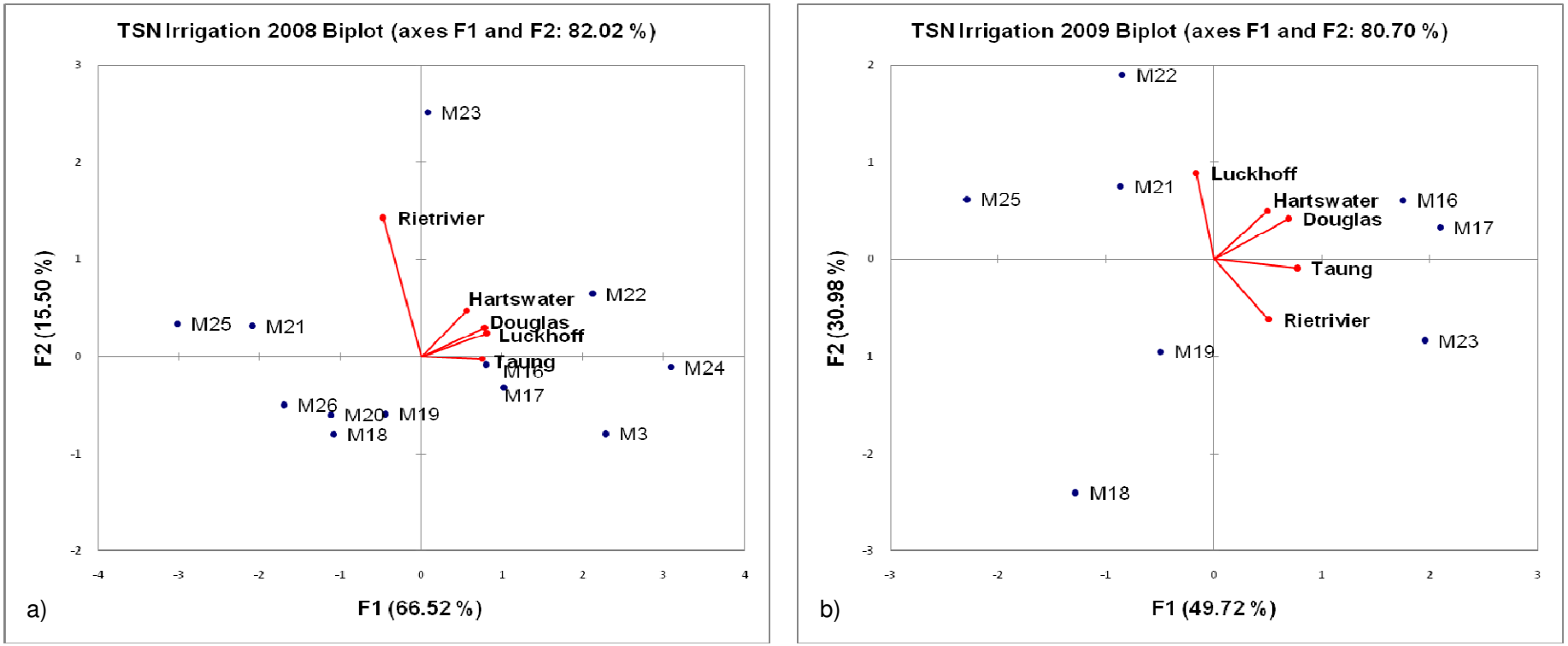


Figure 4.6 PCA biplots for total soluble nitrogen (TSN) of irrigation samples from a) 2008 and b) 2009.

Kolbach Index (KI)

From the dry land PCA biplots (**Fig. 4.7**), it can be seen that M3, M4, M8 and M13 had higher KI for most localities over both seasons, whereas M10 had high KI specifically for Napier in 2008 and 2009. Seasonal differences also occurred; for example, M1 and M7 had high KI for most localities in 2008 but lower KI in 2009. M6 had low KI over both years. As seen in the 2008 biplot M11 and M15 had lower KI than most other lines in 2008 and were not included in the breeding programme in 2009.

Fig. 4.8 indicates that M18, M19 and M25 had low KI over both seasons for the irrigation areas. M22 tended to have a higher KI for Luckhoff in both seasons. Seasonal differences were observed; M21 had lower KI for Hartswater, Douglas and Luckhoff in 2008 and higher KI in 2009 for these localities, whereas M17 had higher KI for Taung, Rietrivier and Luckhoff in 2008, but higher KI for Taung, Hartswater and Douglas in 2009. M16 and M23 were the only lines with a high KI for most localities over both years. M20 and M26 had low KI in 2008 and were not included in the breeding programme in 2009.

Dry land KI showed significant negative correlations ($P < 0.01$) with wort viscosity and β-glucan content, and significant positive correlations ($P < 0.01$) with TSN, FAN, AAL and extract ($P < 0.05$) for the 2008 season (**Table 4.2**). Similar results were observed for the 2009 samples (**Table 4.4**), where KI was negatively correlated ($P < 0.01$) with TN, wort viscosity and β-glucan content, and positively correlated ($P < 0.01$) with extract, FAN and AAL. For the 2008 irrigation samples (**Table 4.3**), KI was negatively correlated ($P < 0.01$) with wort viscosity and positively correlated ($P < 0.01$) with TSN, FAN and wort β-glucan content. Data from the 2009 season showed KI to be negatively correlated ($P < 0.01$) with TN but positively correlated ($P < 0.01$) with TSN, FAN and AAL (**Table 4.5**).

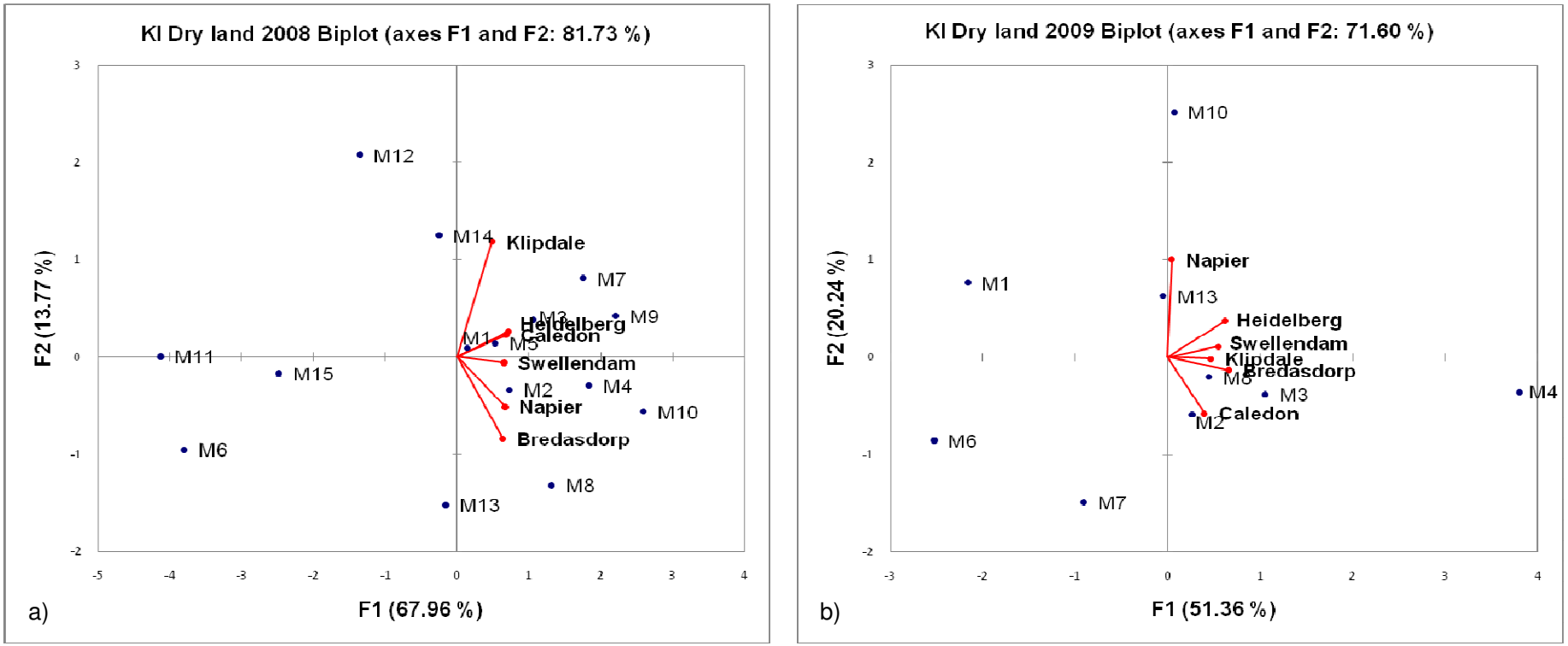


Figure 4.7 PCA biplots for Kolbach Index (KI) of dry land samples from a) 2008 and b) 2009.

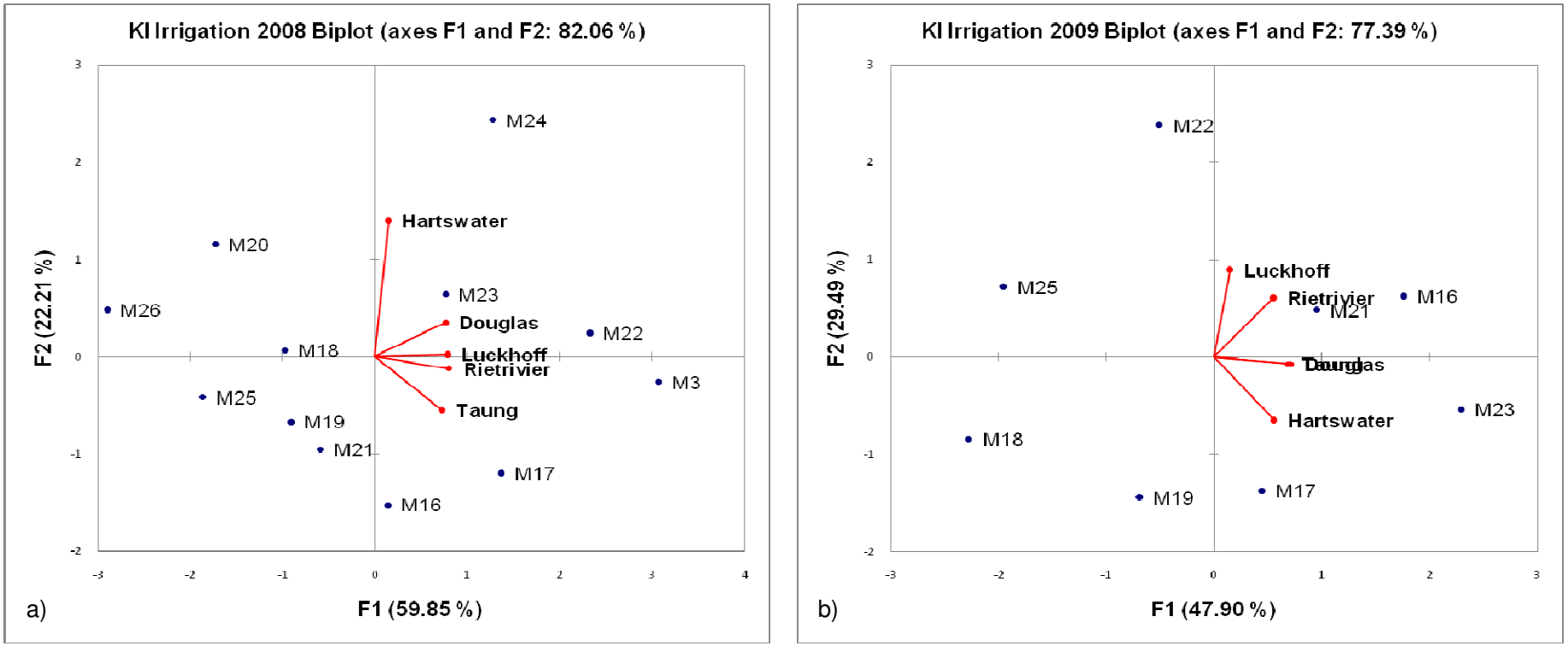


Figure 4.8 PCA biplots for Kolbach Index (KI) of irrigation samples from a) 2008 and b) 2009.

Free amino nitrogen (FAN)

Fig. 4.9 showed that M1, M2, M3, M4, M8 and M10 had high FAN for most dry land localities over both seasons. Seasonal differences were observed for M7, where it had high FAN values for Heidelberg, Swellendam, Klipdale and Caledon for 2008, but low FAN values for these localities in 2009. M11, M12 and M15 had low FAN in 2008 and were not included in 2009. M6 had low FAN for both seasons and might be excluded from the programme if it continues having low FAN values in future seasons.

The PCA biplots for irrigation samples (**Fig. 4.10**) indicated that M16, M17 and M23 had high FAN over both seasons. M16 had especially high FAN for Rietrivier and Douglas. Some seasonal variation was seen; M18 and M19 had low FAN values for Rietrivier and Douglas in 2008 but high values for these localities in 2009. M17 had high FAN for Luckhoff over both seasons. M21 had low FAN for Luckhoff and Hartswater in 2008, but high FAN for these sites in 2009, while M25 had low FAN for most localities over both years. M20 and M26 had low FAN for 2008 and were not included in the breeding programme in 2009.

For the 2008 dry land samples, FAN was negatively correlated ($P < 0.01$) with extract, wort viscosity and β-glucan content and positively correlated ($P < 0.01$) with TN, TSN, KI, and DP (**Table 4.2**). Similar correlation results were observed in 2009, but FAN was also positively correlated ($P < 0.01$) with AAL (**Table 4.4**). FAN of 2008 irrigation samples was negatively correlated ($P < 0.01$) with extract, and positively correlated ($P < 0.01$) with TN, TSN, KI and DP ($P < 0.05$) (**Table 4.3**). Similar results were also observed in 2009, but FAN was also negatively correlated ($P < 0.01$) with β-glucan content (**Table 4.5**).

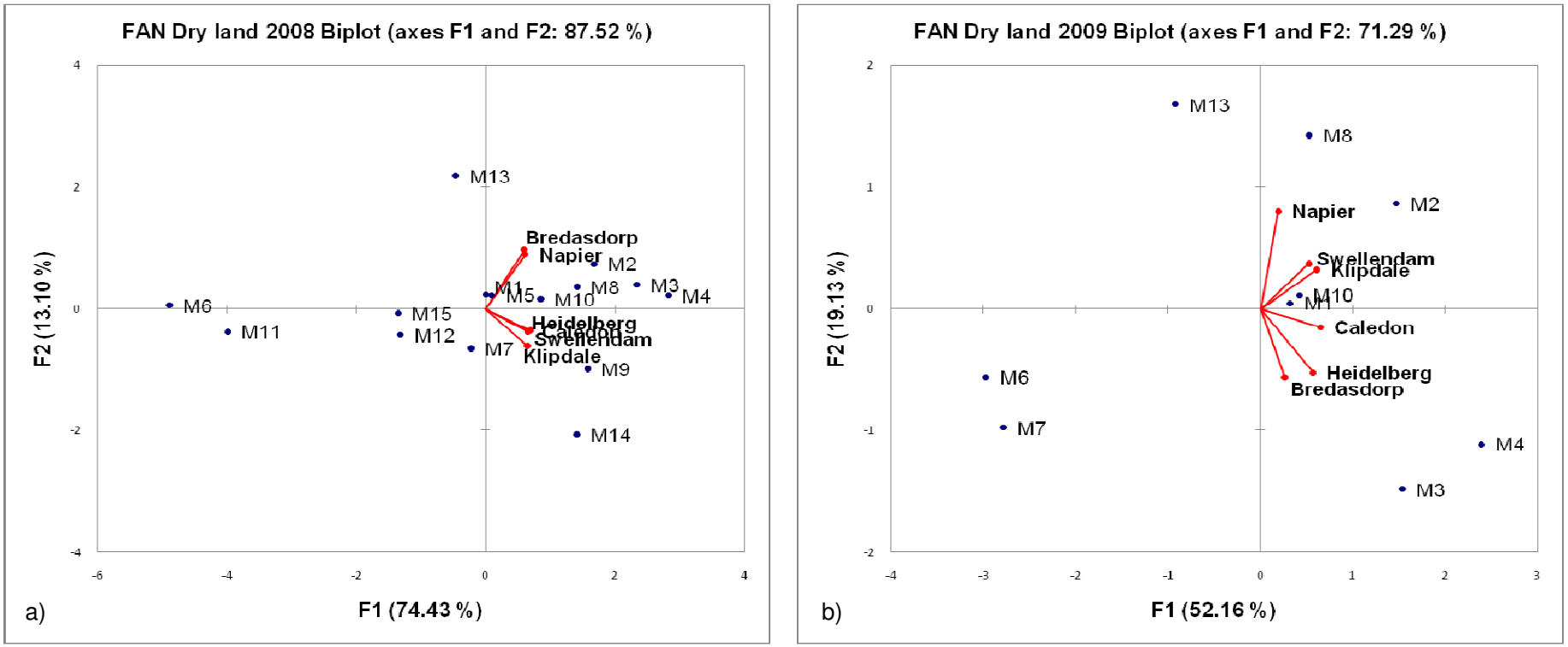


Figure 4.9 PCA biplots for free amino nitrogen (FAN) of dry land samples from a) 2008 and b) 2009.

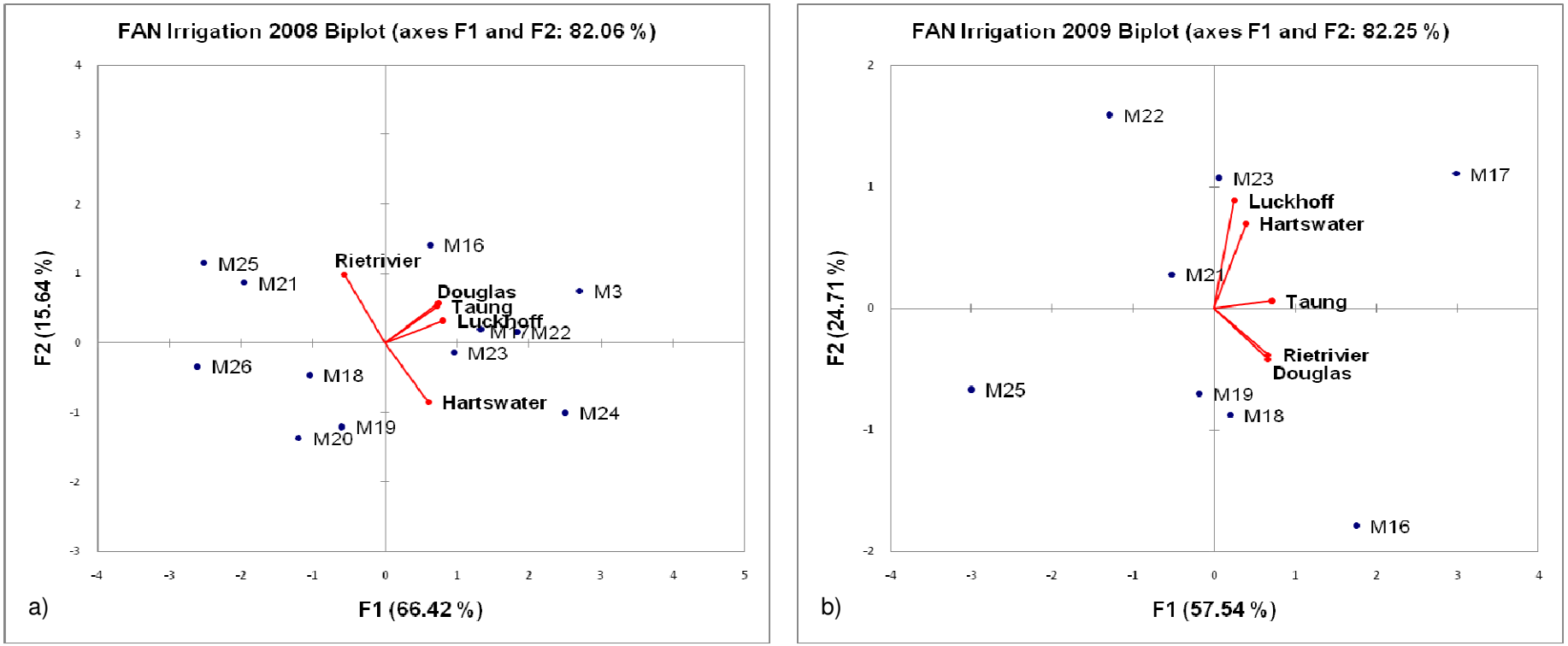


Figure 4.10 PCA biplots for free amino nitrogen (FAN) of irrigation samples from a) 2008 and b) 2009.

Diastatic power (DP)

M1, M7, M8, M10 and M13 had high DP for most dry land locations in both seasons (**Fig. 4.11**), whereas M2, M4 and M6 mostly had low DP. M3 and M7 proved to have especially high DP for Bredasdorp and Klipdale over the two seasons. M11 had very low DP for all localities in 2008 and was not included in the programme in 2009.

PCA biplots for the irrigation samples (**Fig. 4.12**) showed that M17 and M18 had high DP for Luckhoff; M22 and M23 had high DP for Hartswater and M19 for Taung over both seasons. M16, M21 and M25 had high DP for both seasons, although locality differences were seen over the two seasons; M16 had high DP for Luckhoff and Douglas in 2008 but high DP for Douglas, Hartswater and Rietrivier in 2009; M21 had high DP in Hartswater, Rietrivier and Taung in 2008 while higher DP was observed for Luckhoff in 2009; M25 had higher DP for Rietrivier, Taung and Douglas in 2008 and higher DP for Hartswater and Douglas in 2009.

DP for the dry land samples showed negative correlations ($P < 0.01$) with wort viscosity and AAL and positive correlations ($P < 0.01$) with TN, TSN and FAN in the 2008 harvest season (**Table 4.2**). Similar positive correlations were observed in 2009 but DP was negatively correlated ($P < 0.01$) with extract, wort viscosity and β-glucan content (**Table 4.4**). Irrigation samples showed that DP was negatively correlated ($P < 0.01$) with extract, wort viscosity and β-glucan content ($P < 0.05$) but positively correlated ($P < 0.01$) with FAN and AAL in the 2008 season (**Table 4.3**). The 2009 season showed DP to be negatively correlated with extract and positively correlated ($P < 0.01$) with TN, TSN and FAN ($P < 0.05$) (**Table 4.5**).

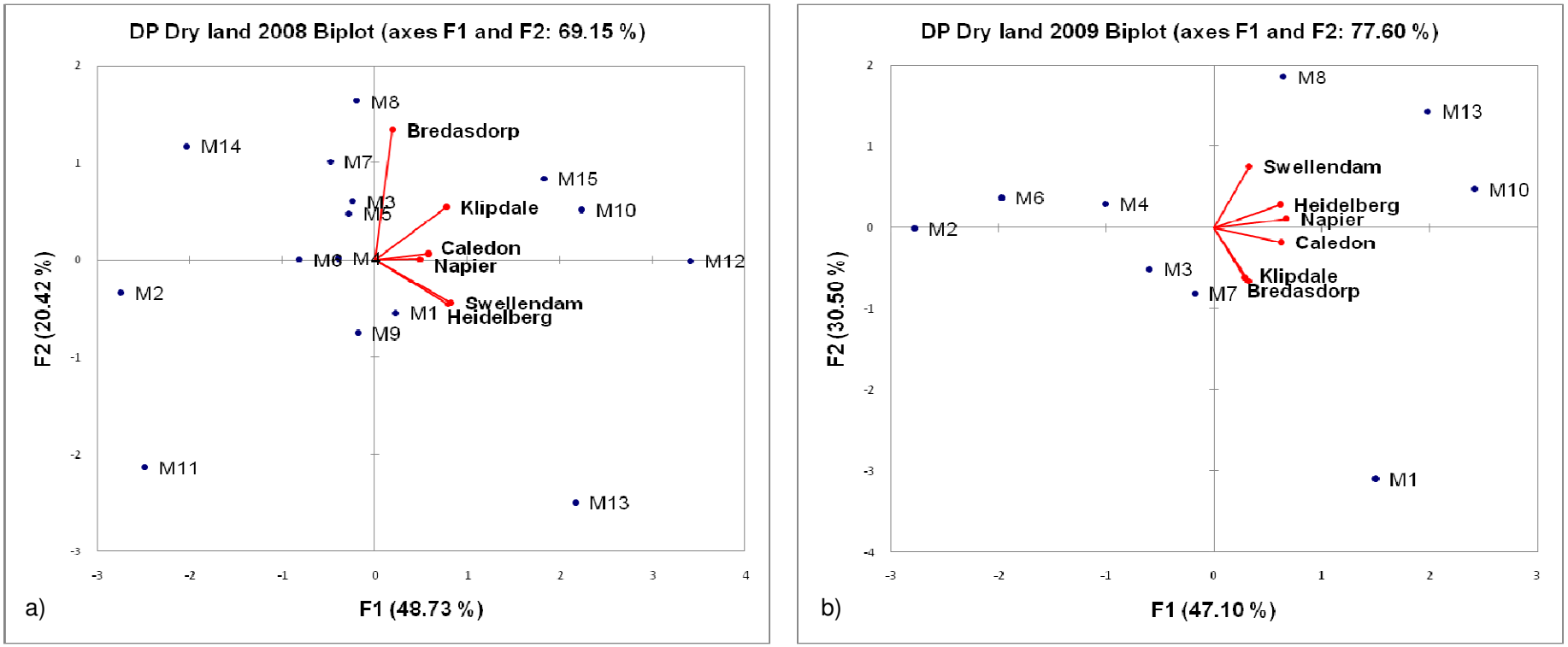
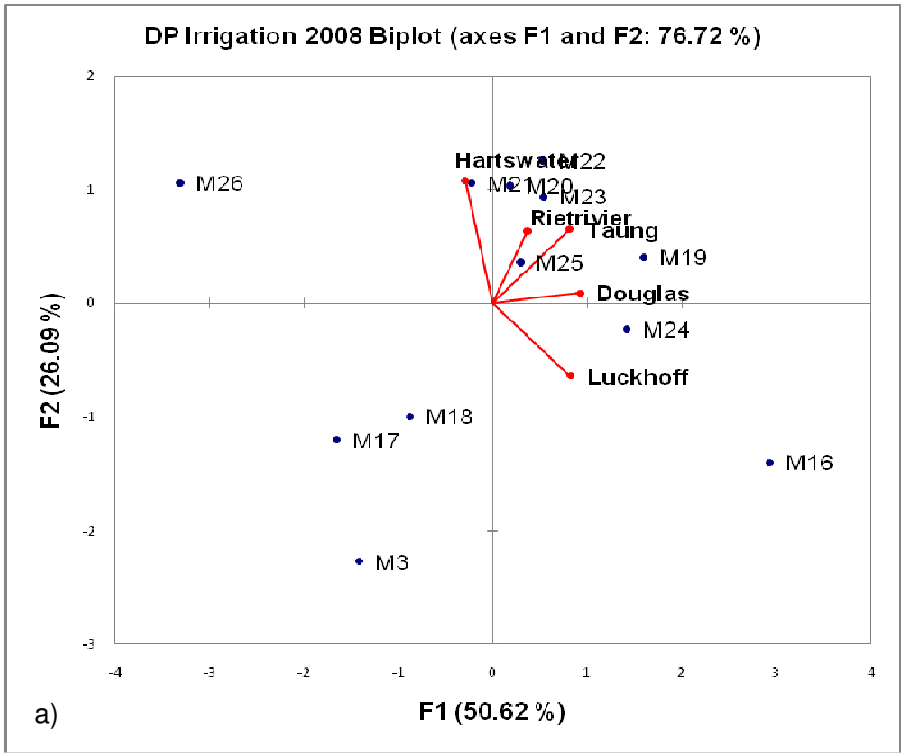


Figure 4.11 PCA biplots for diastatic power (DP) of dry land samples from a) 2008 and b) 2009.



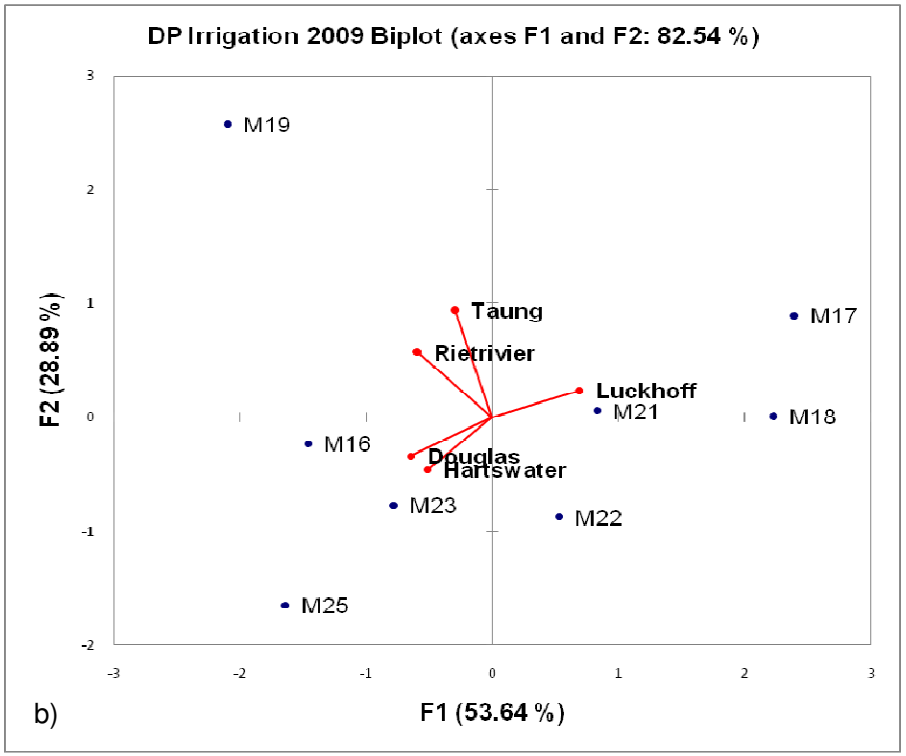


Figure 4.12 PCA biplots for diastatic power (DP) of irrigation samples from a) 2008 and b) 2009.

Wort viscosity

From the PCA biplots for dry land samples (**Fig. 4.13**), it can be seen that most lines had low viscosity over both seasons. M3 and M6 had high viscosity in 2008 and 2009. Seasonal differences were also observed, where M7 and M13 had low viscosity for most localities in 2008 but high viscosity in 2009. Inconsistency was seen over seasons, M1 and M2 had higher viscosity in 2008 and lower viscosity in 2009. M11, M14 and M15 had high viscosity (especially for Bredasdorp) in 2008 and were excluded in 2009.

The PCA biplots for the irrigation areas (**Fig. 4.14**) showed that M3, M20 and M26 had high viscosity for most localities in 2008 and were removed in 2009. M21 and M25 had high viscosity for Rietrivier over both years, whereas M17 had particularly high viscosity for Hartswater and Taung over both years, indicating consistency for these lines and localities over the two seasons. M16 had high viscosity for Hartswater in 2008 but low viscosity for this site in 2009. M18 and M19 had very high viscosity for Hartswater and Rietrivier in 2008 but high values were only seen for Luckhoff in 2009.

Wort viscosity of dry land 2008 samples was negatively correlated ($P < 0.01$) with extract, TSN, KI, FAN, DP and AAL and positively correlated ($P < 0.01$) with wort β-glucan content (**Table 4.2**). In the 2009 season, wort viscosity showed negative correlations ($P < 0.01$) with extract, TN, TSN, KI, FAN, and DP and positive correlations ($P < 0.01$) with extract and wort β-glucan content (**Table 4.4**). For the 2008 irrigation samples, wort viscosity was negatively correlated ($P < 0.01$) with extract, DP, AAL and KI ($P < 0.05$) but positively correlated ($P < 0.01$) with TN and TSN (**Table 4.3**). In 2009 this property was negatively correlated ($P < 0.01$) with AAL only and positively correlated ($P < 0.01$) with TN, TSN and wort β-glucan content (**Table 4.4**).

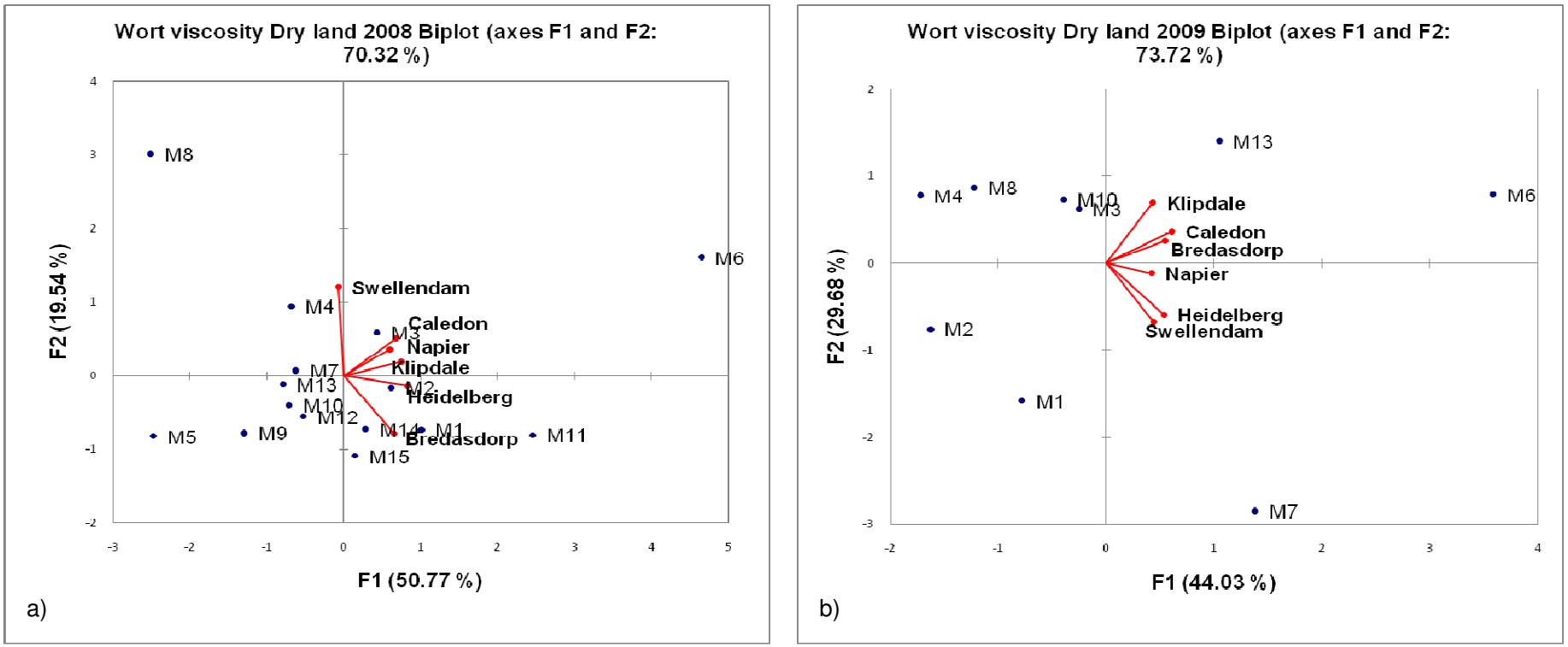


Figure 4.13 PCA biplots for wort viscosity of dry land samples from a) 2008 and b) 2009.

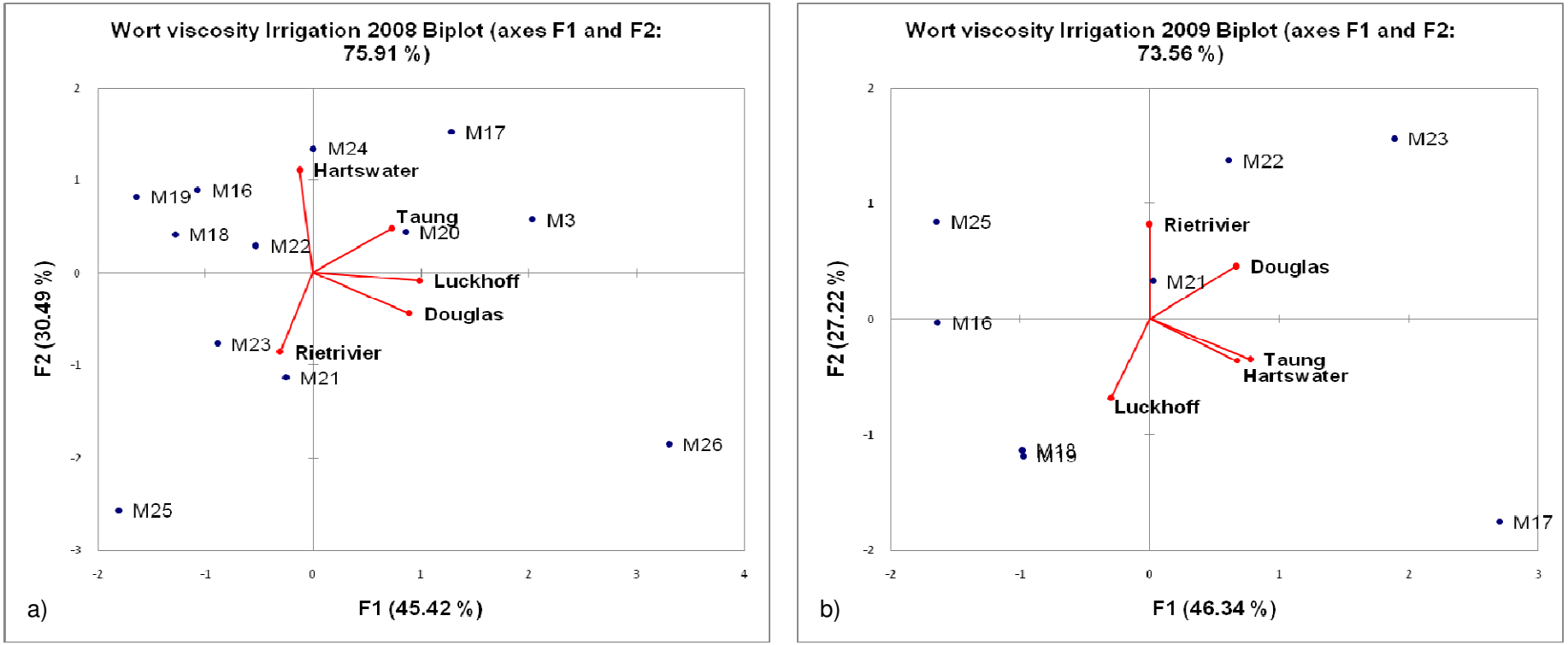


Figure 4.14 PCA biplots for wort viscosity of irrigation samples from a) 2008 and b) 2009.

Apparent attenuation limit (AAL)

M3, M4, M7, M10 and M13 had high AAL for most dry land localities (**Fig. 4.15**) over both seasons, whereas M1, M2 and M8 had low AAL over most localities for the two years. Seasonal and locality dependent differences also occurred; M6 had high AAL specifically for Klipdale and Bredasdorp in 2008 but low AAL for most localities in 2009. M14 and M15 had particularly low AAL in 2008 and were excluded in 2009.

Fig 4.16 indicated that M19 and M21 had high AAL for most irrigation localities over the two seasons, whereas M17 had low AAL for the two seasons. Again, seasonal differences were apparent, where M18 and M23 had low AAL for Douglas and Rietrivier in 2008 but high AAL for these localities in 2009. M22 had high AAL for all localities in 2008 but only had high AAL for Luckhoff in 2009. M25 tended to have high AAL for all localities in 2008 but only for Rietrivier in 2009. M26 mostly had low AAL in 2008 and was excluded in 2009.

AAL for 2008 dry land samples showed negative correlations ($P < 0.01$) with TN, DP, wort viscosity, β-glucan content and TSN ($P < 0.05$), and significant positive correlations ($P < 0.01$) with extract and KI (**Table 4.2**). The 2009 data showed significant negative ($P < 0.01$) correlations for TN and wort β-glucan content and significant positive correlations ($P < 0.01$) with extract, FAN and KI (**Table 4.4**). According to **Table 4.3**, AAL for irrigation samples was negatively correlated ($P < 0.01$) with extract, wort viscosity and β-glucan content and positively correlated ($P < 0.01$) with DP in 2008. In 2009 (**Table 4.5**) AAL was negatively correlated ($P < 0.01$) with TN, TSN, wort viscosity and β-glucan content and only positively correlated ($P < 0.01$) with KI.

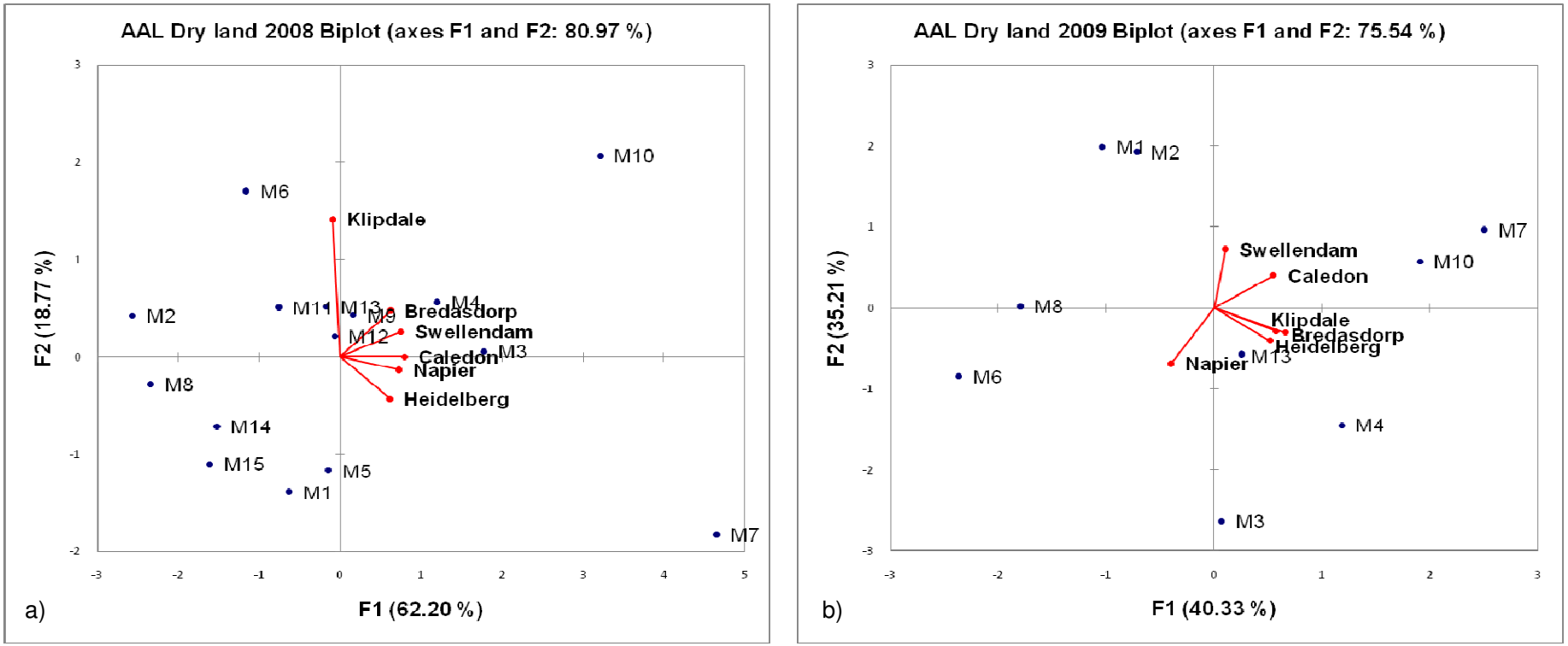


Figure 4.15 PCA biplots for apparent attenuation limit (AAL) of dry land samples from a) 2008 and b) 2009.

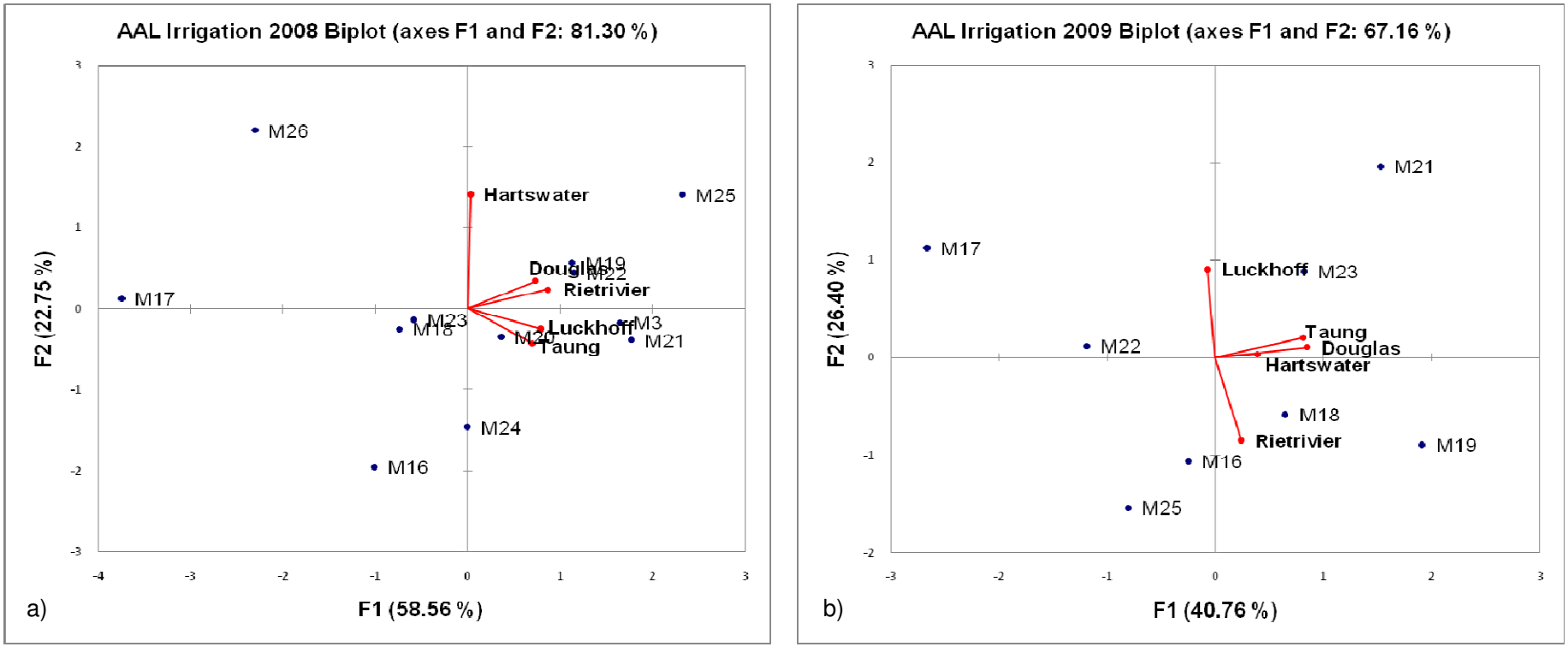


Figure 4.16 PCA biplots for apparent attenuation limit (AAL) of irrigation samples from a) 2008 and b) 2009.

Wort *β-glucan content*

Fig. 4.17 showed that M1 and M7 had high β-glucan levels for most dry land localities over both seasons. M12, M14 and M15 had high levels in 2008 and were excluded in 2009. M13 had high levels for Klipdale, Heidelberg and Swellendam in 2008, but in 2009 it had high levels for Napier, Klipdale and Caledon. Only M2, M3 and M4 had low β-glucan levels over both seasons. M6 had high levels for Napier and Caledon in 2008 but high levels for Heidelberg, Bredasdorp and Swellendam in 2009. M11 had very high β-glucan levels in 2008 and was excluded in 2009.

The PCA biplots for the irrigation samples (**Fig. 4.18**) indicated that M18, M19 and M21 had low β-glucan content for most sites over both seasons. M16 had high levels for Rietrivier and Hartswater in 2008, but high levels for Luckhoff and Rietrivier in 2009. M24 and M26 had high β-glucan levels in 2008 and were excluded in 2009. M22 and M23 had high levels over both years for most localities. M17 and M25 had low β-glucan levels in 2008 but high levels in 2009, especially for Douglas.

As shown in **Table 4.2**, wort β-glucan content for the 2008 dry land samples showed a significant negative correlation ($P < 0.01$) with TSN, KI, FAN and AAL and a positive correlation ($P < 0.01$) with wort viscosity. **Table 4.4** indicates that wort β-glucan content was negatively correlated ($P < 0.01$) with TSN, KI, DP, FAN and AAL and positively correlated ($P < 0.01$) with TN and wort viscosity. Wort β-glucan content for the 2008 irrigation samples was negatively correlated with DP ($P < 0.01$) and AAL ($P < 0.05$) but positively correlated ($P < 0.05$) with TN and KI (**Table 4.3**). For the 2009 season wort β-glucan content was negatively correlated ($P < 0.01$) with FAN and AAL but positively correlated ($P < 0.01$) with TN and wort viscosity (**Table 4.5**).

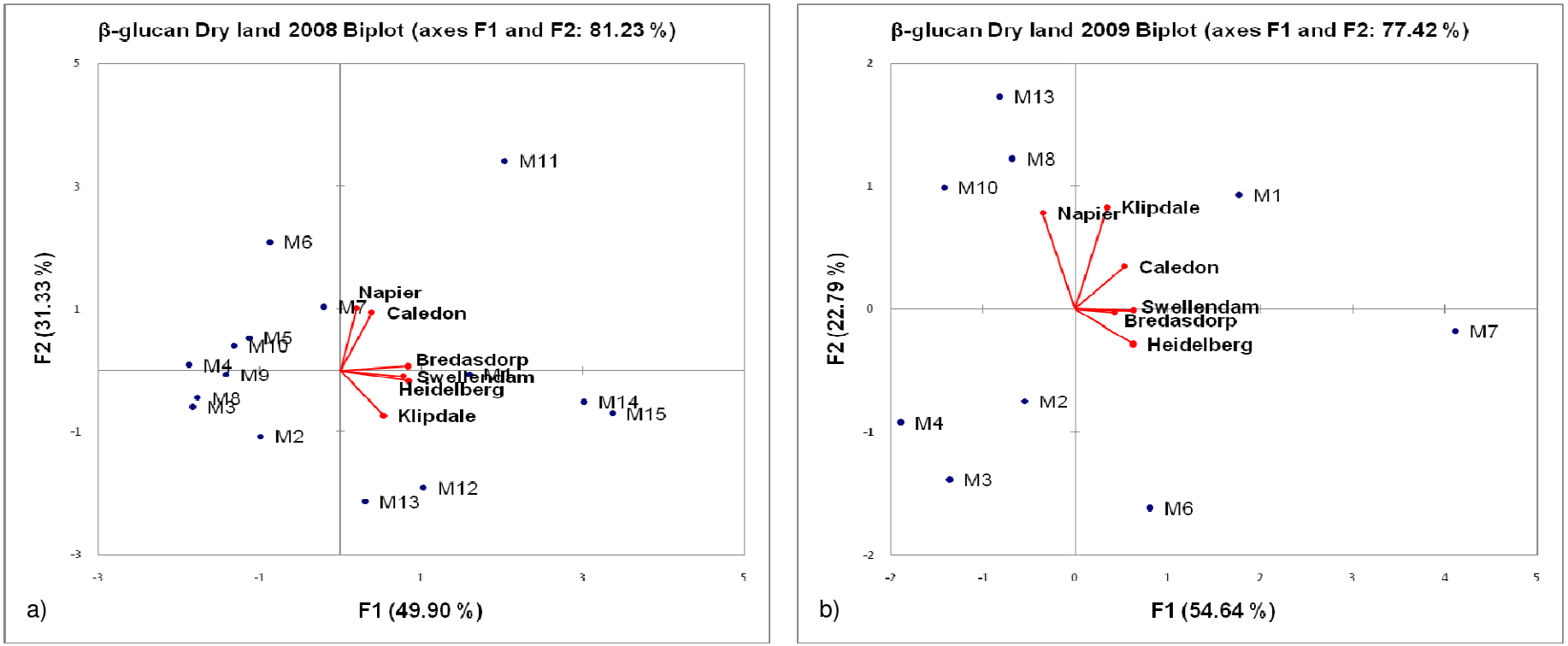


Figure 4.17 PCA biplots for wort β-glucan content of dry land samples from a) 2008 and b) 2009.

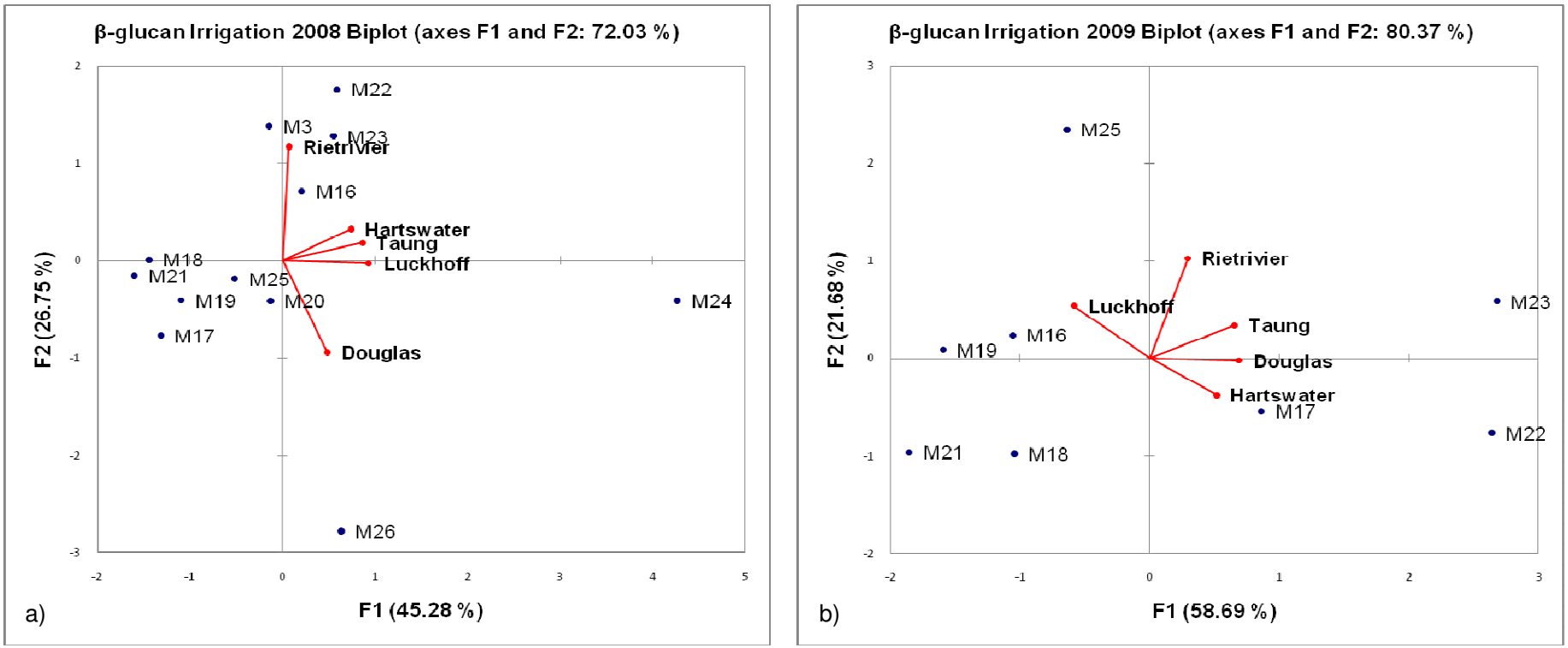


Figure 4.18 PCA biplots for wort β-glucan content of irrigation samples from a) 2008 and b) 2009.

Table 4.2 Correlation matrix for dry land 2008 samples

Property	Extract	TN	TSN	KI	FAN	DP	Viscosity	AAL	β-glucan
Extract	1.00	**-0.51****	**-0.29****	**0.15***	**-0.23****	-0.09	**-0.19****	**0.19****	0.01
TN	**-0.51****	1.00	**0.76****	0.00	**0.68****	**0.50****	0.05	**-0.38****	-0.06
TSN	**-0.29****	**0.76****	1.00	**0.65****	**0.95****	**0.37****	**-0.21****	**-0.12***	**-0.32****
KI	**0.15***	0.00	**0.65****	1.00	**0.68****	-0.01	**-0.40****	**0.27****	**-0.44****
FAN	**-0.23****	**0.68****	**0.95****	**0.68****	1.00	**0.34****	**-0.20****	-0.09	**-0.37****
DP	-0.09	**0.50****	**0.37****	-0.01	**0.34****	1.00	**-0.17****	**-0.26****	-0.07
Viscosity	**-0.19****	0.05	**-0.21****	**-0.40****	**-0.20****	**-0.17****	1.00	**-0.16****	**0.30****
AAL	**0.19****	**-0.38****	**-0.12***	**0.27****	-0.09	**-0.26****	**-0.16****	1.00	**-0.32****
β-glucan	0.01	-0.06	**-0.32****	**-0.44****	**-0.37****	-0.07	**0.30****	**-0.32****	1.00

** significant at P<0.05*

*** significant at P<0.01*

Table 4.3 Correlation matrix for irrigation 2008 samples

Property	Extract	TN	TSN	KI	FAN	DP	Viscosity	AAL	β-glucan
Extract	**1.00**	**-0.47****	**-0.38****	-0.07	**-0.37****	**-0.25****	**-0.27****	**-0.25****	-0.06
TN	**-0.47****	**1.00**	**0.51****	0.10	**0.29****	0.13	**0.30****	-0.08	**0.19****
TSN	**-0.38****	**0.51****	**1.00**	**0.45****	**0.82****	0.13	**0.32****	0.03	0.13
KI	-0.07	0.10	**0.45****	**1.00**	**0.65****	0.04	**-0.18****	0.14	**0.19****
FAN	**-0.37****	**0.29****	**0.82****	**0.65****	**1.00**	**0.21****	0.12	0.11	0.03
DP	**-0.25****	0.13	0.13	0.04	**0.21****	**1.00**	**-0.37****	**0.45****	**-0.19***
Viscosity	**-0.27****	**0.30****	**0.32****	**-0.18***	0.12	**-0.37****	**1.00**	**-0.21****	0.02
AAL	**-0.25****	-0.08	0.03	0.14	0.11	**0.45****	**-0.21****	**1.00**	**-0.16****
β-glucan	-0.06	**0.19***	0.13	**0.19***	0.03	**-0.19***	0.02	**-0.16***	**1.00**

** significant at P<0.05*

*** significant at P<0.01*

Table 4.4 Correlation matrix for dry land 2009 samples

Property	Extract	TN	TSN	KI	FAN	DP	Viscosity	AAL	β-glucan
Extract	**1.00**	**-0.75****	**-0.48****	**0.42****	**-0.27****	**-0.38****	**0.27****	**0.32****	-0.13
TN	**-0.75****	**1.00**	**0.68****	**-0.54****	**0.34****	**0.55****	**-0.28****	**-0.32****	**0.23****
TSN	**-0.48****	**0.68****	**1.00**	0.13	**0.82****	**0.67****	**-0.60****	0.02	**-0.34****
KI	**0.42****	**-0.54****	0.13	**1.00**	**0.35****	0.00	**-0.30****	**0.30****	**-0.55****
FAN	**-0.27****	**0.34****	**0.82****	**0.35****	**1.00**	**0.50****	**-0.49****	**0.23****	**-0.41****
DP	**-0.38****	**0.55****	**0.67****	0.00	**0.50****	**1.00**	**-0.37****	0.15	**-0.30****
Viscosity	**0.27****	**-0.28****	**-0.60****	**-0.30****	**-0.49****	**-0.37****	**1.00**	-0.06	**0.43****
AAL	**0.32****	**-0.32****	0.02	**0.30****	**0.23****	0.15	-0.06	**1.00**	**-0.40****
β-glucan	-0.13	**0.23****	**-0.34****	**-0.55****	**-0.41****	**-0.30****	**0.43****	**-0.40****	**1.00**

** significant at P<0.05*

*** significant at P<0.01*

Table 4.5 Correlation matrix for irrigation 2009 samples

Property	Extract	TN	TSN	KI	FAN	DP	Viscosity	AAL	β-glucan
Extract	**1.00**	**-0.24****	**-0.28****	-0.14	**-0.26****	**-0.31****	-0.06	0.10	-0.16
TN	**-0.24****	**1.00**	**0.69****	**-0.43****	**0.47****	**0.42****	**0.45****	**-0.71****	**0.25****
TSN	**-0.28****	**0.69****	**1.00**	**0.27****	**0.76****	**0.24****	**0.31****	**-0.40****	0.12
KI	-0.14	**-0.43****	**0.27****	**1.00**	**0.25****	-0.14	-0.17	**0.40****	-0.12
FAN	**-0.26****	**0.47****	**0.76****	**0.25****	**1.00**	**0.19***	-0.01	-0.12	**-0.33****
DP	**-0.31****	**0.42****	**0.24****	-0.14	**0.19***	**1.00**	0.00	0.15	0.04
Viscosity	-0.06	**0.45****	**0.31****	-0.17	-0.01	0.00	**1.00**	**-0.39****	**0.55****
AAL	0.10	**-0.71****	**-0.40****	**0.40****	-0.12	0.15	**-0.39****	**1.00**	**-0.29****
β-glucan	-0.16	**0.25****	0.12	-0.12	**-0.33****	0.04	**0.55****	**-0.29****	**1.00**

** significant at P<0.05*

*** significant at P<0.01*

Discussion

A number of researchers have reported on the effect of environment, genotype as well as season on grain quality, and even though results differed, all studies concluded that these influences were important factors in the quality of malting barley (Eagles *et al*., 1995; Molina-Cano *et al*., 1997; Oscarsson *et al*., 1998; Kaczmarek *et al*., 1999). **Extract** indicates the maximum soluble yield obtained from a specific malt (Anger *et al*., 2009). The higher the extract the more soluble the material and therefore maltsters demand a high extract value (Kotze, 2009). The extract values of dry land samples indicated consistency over seasons for lines high in extract (M7, M8 and M13) which indicate potential good malting cultivars. A considerable number of localities had higher extract in the irrigation areas (M16, M17, M18, M19, M21, M22 and M25), possibly due to more controlled environmental conditions in these areas and higher quality of the end grain (F. Potgieter, South African Barley Breeding Institute (SABBI), Caledon, South Africa, Personal Communication, 2009). Lines with consistently low extract over both seasons (M1, M3 and M10) can be removed from the programme if they continue having low extract values (as was the case with M15 and M11 which had very low extract values for most sites in 2008 and were subsequently removed from 2009 trials). Seasonal variation was also apparent for lines from both the dry land (M2, M6 and M4) and irrigation areas (M23) and a third season of data would be needed to determine if these lines would deliver consistent quality as commercial cultivars. Consistently high extract values were observed in the dry land areas for Napier and Klipdale indicating that these localities were possibly most suited for growing barley with higher extract potential. M18 had consistently high extract in Rietrivier (irrigation), whereas M23 had low extract for this location over both seasons indicating that extract was also dependent on environmental conditions. This is confirmed by analysis of variance (ANOVA) results of previous researchers, where the interaction of environment was significant ($P \leq 0.01$) for extract (Kaczmarek *et al*., 1999). Genotype and locality also had

significant ($P \leq 0.05$) effects on extract (Molina-Cano *et al.*, 1997), while seasonal differences ($P \leq 0.05$) and cultivar ($P \leq 0.01$) had a significant effect on extract (Eagles *et al.*, 1995).

The presence of low **TN** content in barley indicates potential to provide malt of high extract (Foster *et al.*, 1967; Arends *et al.*, 1995; Eagles *et al.*, 1995; Molina-Cano *et al.*, 1997) but most lines had high TN content for the dry land and irrigation areas. M2 had especially high TN content for most dry land sites which is not ideal for malting barley. These lines may be removed from the breeding programme in future seasons (i.e. M14 and M15 had very high TN in 2008 for the dry land areas while M24 had high TN for the irrigation areas; these lines were therefore omitted from the 2009 trials). M7 and M21 were the only lines with low TN for most locations over both seasons in the dry land and irrigation areas, respectively, and can be considered as potential good malting cultivars. The biplots showed that seasonal differences had a large impact on TN content for dry land (Swellendam, Klipdale and Napier) and irrigation sites (Hartswater, Douglas, Rietrivier, Luckhoff and Taung). Therefore, more information from subsequent seasons is needed to make an informed decision as to which lines have consistent good malting potential over localities. M8 and M3 had consistently higher TN for Heidelberg over both years, and M1 for Caledon, indicating that these sites may have a negative influence on TN. These results are confirmed by previous results where genotype did not have a significant effect on TN, but the effect of location ($P \leq 0.05$) (Molina-Cano *et al.*, 1997) or environment ($P \leq 0.01$) (Kaczmarek *et al.*, 1999) and season ($P \leq 0.05$) (Molina-Cano *et al.*, 1997) was significant.

TSN is an important property for maltsters as it is a measure of the amount of nitrogenous material in wort (Hough, 1991). This nitrogenous material is a food source for yeast during the fermentation process and high quality barley for malting requires low amounts of TSN (Pollock, 1962). A number of dry land lines (M2, M3, M4, M8 and M10) had high TSN for both seasons whereas fewer irrigation lines (M16, M17 and M23) had high TSN over both years. This is an indication of poor malting quality and these lines should possibly be removed from the breeding programme like M14, which had high TSN in 2008 and was not included in 2009. Consistently high TSN values were observed for Napier, Heidelberg and Rietrivier over both years, indicating that this property was negatively influenced by these localities, although a third season would be needed to confirm these results. M6, M7 and M13 had the required low TSN for most dry land localities for both seasons, while M18, M19, M21 and M25 had low TSN for irrigation localities. As was the case with extract, more irrigation localities delivered the ideal TSN values, which confirm that controlled irrigation conditions result in higher barley quality. Seasonal differences were observed for both the dry land and irrigation areas, indicating that a third season of GxE data should be studied before these lines are removed or progressed to the next stage of the breeding programme.

The **KI** relates TSN to TN on a percentage basis and a high KI indicates protein modification has proceeded to a desired extent (Bamforth & Barclay, 1993). Several lines (M3, M4, M8 and M13) had higher KI values for most dry land localities over both seasons, whereas M16 and M23

were the only lines that had high KI for most irrigation localities; all of these lines had acceptable KI for good malting quality. M10 had high KI specifically for Napier in 2008 and 2009, M22 tended to have a higher KI for Luckhoff in both seasons, which indicated that these sites may have a positive influence on KI. Seasonal variation occurred in both regions; for example, M1 and M7 had high KI for most dry land localities in 2008 but lower KI in 2009, whereas M21 had lower KI for Hartswater, Douglas and Luckhoff in 2008 and higher KI for these localities in 2009. Because KI is a ratio of TN to TSN and seasonal differences were observed for these properties, it is to be expected that the KI will also vary over consecutive seasons. Poor malting behaviour was also observed (M6, M18, M19 and M25) over both seasons and these lines could be removed from the programme if it continues having low KI. These results compare well with ANOVA data from previous studies, which showed genotype, location and year had significant ($P \leq 0.05$) effects on KI (Molina-Cano *et al.*, 1997).

Yeast fermentation is limited by **FAN** (e.g. amino acids) and therefore brewing requires threshold levels of usable nitrogenous material for metabolic purposes (Bamforth & Barclay, 1993; Kotze, 2009). For the dry land areas, M1, M2, M3, M4, M8 and M10 had high FAN for most localities over both seasons, while M16, M17 and M23 had high FAN for the irrigation areas, indicating that these lines had good FAN levels. M6 and M25 had low FAN for both seasons in the dry land and irrigation sites, respectively, and might be excluded from the programme if it continues having low FAN values in future seasons. Locality dependency was observed for irrigation samples where M16 had especially high FAN for Rietrivier and Douglas and M17 for Luckhoff in both seasons, and indicates that the irrigation conditions in these sites have a positive influence on the FAN of these lines. Seasonal differences were apparent for this property, i.e. M7 had high FAN values for Heidelberg, Swellendam, Klipdale and Caledon for 2008, but low FAN values for these localities in 2009, M18 and M19 had low FAN values for Rietrivier and Douglas in 2008 but high values in these localities in 2009. M21 had low FAN for Luckhoff and Hartswater in 2008, but high FAN for these sites in 2009. This information shows that breeders need data from additional seasons for final evaluation of these lines in terms of the FAN content.

DP reflects the combined activity of four starch reducing enzymes (α-amylase, β-amylase, α-glucosidase and limit dextrinase) that degrade starch to simpler fermentable sugars and therefore, the DP of good malting barley should be sufficiently high(Duffus & Cochrane, 1993; Shewry & Darlington, 2002; Kotze, 2009). For the dry land areas, M3 and M7 proved to have especially high DP for Bredasdorp and Klipdale over the two seasons which indicated that these sites had a positive influence on DP. The required high DP was observed for a number of lines (M1, M7, M8, M10 and M13) in most dry land localities as well as irrigation localities (M16, M21 and M25). DP of the irrigation lines proved to be more dependent on specific localities (M17 and M18 had high DP for Luckhoff while M22 and M23 had high DP for Hartswater and M19 for Taung over both seasons) but locality differences were also observed over the two seasons (M16 had high DP for Luckhoff and Douglas in 2008 but high DP for Douglas, Hartswater and Rietrivier in 2009; M21 had

high DP in Hartswater, Rietrivier and Taung in 2008 while higher DP was observed for Luckhoff in 2009; M25 had higher DP for Rietrivier, Taung and Douglas in 2008 and higher DP for Hartswater and Douglas in 2009) which indicates that, despite the controlled irrigation environments, DP was still highly influenced by season. ANOVA results have indicated that seasonal differences had a significant effect on DP ($P \leq 0.01$) (Eagles *et al.*, 1995). Similar results were reported for effect of genotype and location on DP, both of which proved to have an influence on DP (Arends *et al.*, 1995).

The **viscosity** of wort provides information about the degree of malt modification (Pollock, 1962) and is a measure of the breakdown of β-glucans during malting (Kotze, 2009b), where high β-glucan levels in malt is a result of incomplete cell wall degradation (Duffus & Cochrane, 1993). Maltsters demand malting barley with low β-glucan levels and therefore low viscosity. Most dry land lines had low viscosity over both seasons and indicate good malting quality, whereas high viscosity was observed for irrigation localities. M21 and M25 had high viscosity for Rietrivier over both years, whereas M17 had particularly high viscosity for Hartswater and Taung over both years, indicating some consistency for these lines and localities to deliver high viscosity. This was confirmed by ANOVA results which showed that genotype and location had a significant ($P \leq 0.05$) effect on viscosity (Molina-Cano *et al.*, 1997). Seasonal differences were observed for both regions, where lines had low viscosity for most localities in 2008 but high viscosity in 2009, or higher viscosity in 2008 and lower viscosity in 2009. These results indicated that viscosity is not only influenced by locality, but also season and that more data is needed to determine if these lines will deliver consistent quality. For the dry land areas M3 and M6 had high viscosity over both seasons which is not ideal for a malting cultivar and these lines may be removed from the programme (such as M11, M14 and M15 which had high viscosity, especially for Bredasdorp, in 2008 and were excluded in 2009).

AAL refers to the percentage of extract converted to alcohol during fermentation and is indicative of the amount of alcohol that can be obtained from wort (Kotze, 2009). M3, M4, M7, M10 and M13 had high AAL (indicating good malting quality) for most dry land localities over both years, whereas M1, M2 and M8 had consistently low AAL (indicating poor malting quality). Results indicated that only M19 and M21 had high AAL for most irrigation localities over the two seasons, whereas M17 had low AAL over the two seasons; this indicated poor malting potential and the controlled irrigation environments did not result in better AAL values. Seasonal differences between localities were apparent for both the dry land and irrigation areas, indicating that AAL was susceptible to environmental changes. A third season of GxE data would be needed for final evaluation of these lines. Similar results were reported in literature; genotype and season had a significant effect ($P \leq 0.05$) on AAL while the effect of location was not significant (Molina-Cano *et al.*, 1997).

β-glucans are the major components of the endosperm cell wall (Fincher & Stone, 1993). High β-glucan levels in a malt sample indicate incomplete cell wall degradation and diminished

mobilization of the starch-protein matrix. This results in lower malt extract values and lower nutrient availability for fermentative growth by yeast during brewing (Duffus & Cochrane, 1993). High β-glucan levels were observed for most dry land (M1 and M7) and irrigation localities (M22 and M23) over both seasons, and since maltsters demand low β-glucan levels, these lines will be removed if they continue to have high levels in future seasons. This property also proved to be highly dependent on season, since several lines delivered high β-glucan levels one year, but lower levels the next year (i.e. M17 and M25 had low β-glucan levels in 2008 but high levels in 2009 (especially for Douglas) and M16 had high levels for Rietrivier and Hartswater in 2008, but high levels for Luckhoff and Rietrivier in 2009). The effect of cultivar and environment had previously been assessed and revealed that cultivar was the most significant factor affecting β-glucan content; while environmental effects were found to be less important (Oscarsson *et al.*, 1998). Good malting behaviour (low β-glucan content) was observed for a number of lines in the dry land (M2, M3 and M4) and irrigation (M18, M19 and M21) regions possibly indicating that these lines had genetically low β-glucan content.

M7 showed similar behaviour for positively correlated properties TN, TSN, KI, DP and FAN ($P < 0.01$), while M4 and M7 showed similar behaviour for viscosity and β-glucan content, which are significantly positively correlated ($P < 0.01$). Differences were observed for correlations between most malt properties over seasons i.e. in 2008 no significant interaction was observed for extract and DP but in 2009 these properties were significantly negatively correlated ($P < 0.01$). This indicated that season had an effect on malting properties. However, FAN delivered relatively consistent results over seasons with regard to growing conditions when compared with the other malt properties; FAN may possibly be influenced by genotype rather than season. Similar correlation results as those summarised in **Tables 4.2 – 4.5** have been reported in literature; extract and viscosity showed a significant negative correlation ($P \leq 0.05$) (Molina-Cano *et al.*, 1997), as was the case with these properties for dry land and irrigation 2008 samples. Extract and KI was significantly positively correlated ($P \leq 0.01$) (Molina-Cano *et al.*, 1997), similarly to dry land 2009 samples. TN showed a significant negative correlation with extract ($P < 0.01$) for all samples types over both seasons; extract was negatively correlated with DP ($P < 0.01$) for irrigation 2008 and 2009 samples but only for dry land 2009 samples. Similar results have been reported in literature where nitrogen and DP showed significant negative correlations with extract ($P \leq 0.01$) (Den Hartog & Lambert, 1953; Foster *et al.*, 1967; Rutger *et al.*, 1967; Arends *et al.*, 1995; Eagles *et al.*, 1995; Molina-Cano *et al.*, 1997). Nitrogen also showed a consistent positive correlation ($P \leq 0.01$) with DP (Den Hartog & Lambert, 1953; Foster *et al.*, 1967; Rutger *et al.*, 1967; Arends *et al.*, 1995) as was observed in this thesis for dry land (2008 and 2009) and irrigation (2009) samples ($P < 0.01$). Viscosity and TN have been reported to have a significant positive correlation ($P \leq 0.01$) (Molina-Cano *et al.*, 1997) which was also observed for the irrigation areas in 2008 and 2009 but only for dry land 2009 samples ($P < 0.01$). TN also showed a significant negative correlation ($P \leq 0.01$) with KI (Molina-Cano *et al.*, 1997) which was only seen for the 2009 harvest in this study. A

significant positive correlation ($P \leq 0.05$) also existed between KI and AAL (Molina-Cano *et al.*, 1997) and similar results were observed for the dry land 2008 and 2009 samples, but only the irrigation 2009 samples.

A good commercial malting cultivar should have the desired high or low levels for as many malting properties as possible, in order to deliver malt of high quality. Taking all nine malt properties into consideration, M7 proved to be a potential good malting cultivar for the dry land areas since it had low TN and TSN as well as high extract, DP and AAL. M13 had high KI, extract, DP and AAL and low TSN whereas M3 had high KI, FAN, AAL and low β-glucan content. M4 had high KI, FAN, AAL but also low viscosity and β-glucan content. M8 had high KI, extract, DP, FAN and low viscosity while M10 had high KI, FAN, DP and AAL and low viscosity. For irrigated areas, M18 and M19 had low TSN, β-glucan content and viscosity and high extract and DP, while M19 also had high AAL. M17 had high KI, FAN, extract and DP. M21 had low TSN, and β-glucan content and also high extract and AAL. The behaviour of these lines with regard to all malt properties should therefore be considered when evaluating lines for possible use as commercial cultivars.

Locality clustering was observed, i.e. for the dry land areas, Bredasdorp and Napier were consistently situated closely together in 2008 for Extract, TN, TSN, KI and FAN indicating similar environmental properties, whereas Napier was situated away from the other localities in 2009 for extract, TSN, KI, FAN. For the 2008 irrigation areas, Rietrivier and Hartswater were in their own cluster away from the other localities for the properties extract, viscosity and FAN. Hartswater tended to be situated away from the other localities for TN, KI and AAL, whereas Rietrivier was situated on its own for TSN. In 2009, all irrigation localities seemed to be relatively separated from each other. This shows that even though the irrigation localities are controlled environments, seasonal differences can still be observed within localities.

A number of lines were consistently located close together in the biplots over the two growing seasons for the dry land and irrigation areas suggesting that these lines may have very similar genetic properties and were similarly influenced by season. For the dry land areas, M3 and M4 were the only two lines consistently located closely together; in 2008 for the properties extract, TSN, KI, FAN, DP, AAL, viscosity as well as β-glucan content. In 2009, these two lines were positioned together for extract, TSN, FAN, DP, AAL as well as β-glucan content. In the biplots for irrigation areas, M18 and M19 were situated close together for the properties extract, TSN, KI, FAN, viscosity and β-glucan content in 2008 and for the properties TN, KI, FAN, AAL and β-glucan content in 2009. M22 and M23 were located close together for extract, TN, KI, FAN, DP, viscosity and β-glucan content in 2008, but only for FAN, DP, viscosity and β-glucan content (which showed seasonal differences occurred). Since the irrigation environments were more controlled than the dry land environments, it was to be expected that more lines would behave similarly in the irrigation areas than in the dry land areas.

Several line-locality pairings were observed where lines had consistently high values for specific properties in specific localities for both the 2008 and 2009 seasons in the dry land and irrigation areas. The irrigation areas delivered more consistent results over the two harvest seasons, i.e. more lines had consistent results for the same locality, because of the controlled irrigation conditions in all irrigation localities. This indicated that these localities tend to deliver consistently high values for these properties over seasons and depending on the desired level for each property (for example high extract values are demanded by brewers, but low TN values are needed for good malting quality) breeders should also take locality influences into consideration.

Conclusion

Using PCA biplots GxE interactions were interpreted, which proved to be effective in the evaluation of genotype responses in a number of localities over consecutive seasons. The data from the two seasons showed that a number of lines had consistent results for a given property over seasons and could give an indication of genetically poor or good malting barley. However, differences were also seen over seasons where a line had a low value for a property in 2008 but a high value for that same property in 2009, which can be attributed to environmental changes over subsequent seasons. As a result, the GxE interaction should be studied over more than two seasons to determine if there is a consistency in quality. Elite trials in the breeding programme (Chapter 2.3), where fewer lines are tested over a wide range of localities, are carried out over three years and would be an adequate time span to assess the seasonal and locality effects on breeding lines. Such evaluation would allow breeders to make a conclusion as to which lines should make the transition to commercial cultivar and which localities tend to enhance the quality of certain lines. Ideally, field replicates should not be bulked when malting quality is evaluated, since this ignores that variation is also present within a single field trial and not just over localities or seasons and a more objective assessment of the GxE interaction can be achieved by retaining field replicates.

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Chapter 5

General discussion and conclusion

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The production of malt is dependent on the initial raw material, i.e. the raw barley grain (Savin & Molina-Cano, 2002), and therefore evaluation of barley quality traits are vital in improving barley quality through breeding (Ullrich, 2002). Evaluation of these barley, as well as malt, quality characteristics requires large numbers of lines to be evaluated at early stages of the breeding programme (Meredith *et al*., 1962; Henry, 1985a; Savin & Molina-Cano, 2002). Cultivar choice is the principal economic decision for farmers, and consequently, knowledge of the malting quality of commercial cultivars is essential. Near infrared (NIR) analysis in reflectance mode is an ideal technique for quality evaluation; it allows breeders to conduct rapid tests non-destructively on small samples of grain for early generation quality evaluation where limited seed is available (Woodcock *et al*., 2008). Although various studies on the prediction of malt quality from raw (unmalted) barley grain have been carried out in other parts of the world, no study has to date been performed on malting barley in a South African breeding programme.

Barley samples (n = 2082; 39 cultivars from 16 localities) were obtained from the South African Barley Breeding Institute (SABBI) 2008 breeding trials, grown either under irrigation (n = 732) or dry land (n = 1350) conditions, while barley samples (n = 535; 25 cultivars) were obtained from 13 localities for the 2009 season from the irrigation (n = 178) and dry land (n = 357) areas. Reference data for plumpness and moisture were determined from raw barley samples. Replicates from field trials were bulked; samples were malted and reference values for extract, total nitrogen (TN), total soluble nitrogen (TSN), Kolbach Index (KI), free amino nitrogen (FAN), diastatic power (DP), wort viscosity, apparent attenuation limit (AAL) and wort β-glucan content were determined. Reference values were used in the development of NIR calibration models for prediction of these properties from both whole grain and ground South African barley, using three NIR instruments (Büchi NIRFlex N-500, Bruker MPA and Büchi NIRLab N-200).

Principal component analysis (PCA) of the Bruker MPA spectra indicated that NIR spectra clustered with respect to cultivation environment (i.e. dry land and irrigation). PCA score plots confirmed that irrigation samples had more consistent quality over localities due to more controlled environmental conditions, when compared to dry land samples. Only in the more variable dry land sample set was clustering based on growth locality distinguishable. Overall, irrigation samples were of higher quality than dry land samples, largely due to the higher plumpness and more ideal TN values associated with irrigation samples. NIR spectroscopy in combination with PCA can therefore allow for quick quality evaluation based solely on spectral data, and allow breeders to distinguish between samples with higher and lower quality.

For the 2008 season, three spectrometers were used for evaluation. Whole grain spectra were recorded with Bruker MPA and Büchi NIRFlex N-500 spectrometers, while whole grain and flour

spectra were recorded with the Büchi NIRLab N-200 spectrometer. The Büchi NIRLab N-200 was used for recording spectra of samples from the 2009 season. PLS models were developed for the 2008 season as well as a combination of samples from the 2008 and 2009 harvest seasons (only for moisture, TN and TSN). A number of pretreatment techniques were applied; models from the Büchi instruments were validated using test sets as well as variable selection through uncertainty testing with segmented cross-validation (Martens & Martens, 2000). The Bruker MPA data was evaluated by test set validation only.

Moisture content was predicted with greater accuracy when based on flour rather than whole grain. Moisture predictions from Büchi NIRFlex N-500 data for dry land whole grain samples were poor but the Bruker MPA dry land whole grain model was acceptable for rough screening (r^2 = 0.53). The best results for moisture content prediction from dry land whole grain samples were obtained for Büchi NIRLab N-200 data with the application of selected spectral variables (r^2 = 0.60). Prediction models developed for irrigation whole grain samples from these instruments were unacceptable. Good flour models were developed from Büchi NIRLab N-200 data (irrigation r^2 = 0.69; dry land r^2 = 0.76) and would be acceptable for rough screening purposes. Moisture content determinations from whole grain barley are well established in literature (r^2 = 0.94 – 0.96) (Downey, 1985; Halsey, 1987; Sohn *et al.*, 2008) and flour predictions are even more accurate (r^2 = 0.98, r^2 = 0.99) (Downey, 1985; Henry, 1985b). Results from this study did not compare well to literature reports, due to the smaller sample ranges utilised compared to those used by previous researchers. Flour samples predicted better than whole grain samples because flour spectra were recorded immediately before moisture determinations were performed, whereas the whole grain samples were scanned a few months later which may have resulted in moisture losses during the storage period. Grinding of wholegrain barley for moisture determinations also resulted in moisture losses.

Plumpness was poorly predicted in most cases except for the Büchi NIRLab N-200 data for irrigation whole grain samples (r^2 = 0.52; r^2 = 0.56 with variable selection). The irrigation flour model for this instrument resulted in an r^2 of 0.50. Although these results were acceptable for rough screening, better results were obtained for prediction from whole grain barley in literature (r^2 = 0.83) (Edney *et al.*, 1994). Poor results for the prediction of plumpness were expected. Plumpness refers to the shape of the kernel, and despite increased plumpness often being associated with increased starch content, this parameter was expected to largely manifest as a physical rather than chemical response within NIR spectra. This expectation was confirmed by comparison of flour and whole grain calibration results; where this shape information was removed by grinding, less accurate prediction models were generated.

Extract prediction delivered good calibrations for the Büchi NIRFlex N-500 instrument for irrigation whole grain samples (r^2 = 0.60; r^2 = 0.69 with variable selection). The Bruker MPA model for irrigation samples (r^2 = 0.55) was acceptable for screening purposes and similar results were

obtained for dry land whole grain samples with the Büchi NIRLab N-200 (r^2 = 0.55; r^2 = 0.62 with variable selection). Models developed for irrigation flour samples from Büchi NIRLab N-200 data were also acceptable for rough screening purposes (r^2 = 0.55; r^2 = 0.58 with variable selection), while the poor results for dry land samples (r^2 = 0.24 – 0.39) are due to the small range (78.4 - 83.4%) in reference values obtained. The range of samples needs to be expanded in order to obtain acceptable calibrations. Results from this study did not compare well with that of previous researchers who developed promising calibrations for predicting the extract of whole grain (r^2 = 0.78 - 0.85) (Halsey, 1987; Li *et al*., 1995; Black & Panozzo, 2001) and ground barley (r^2 = 0.77 - 0.96) (Henry, 1985b; McGuire, 1982; Tragoonrung *et al*., 1990). This property is highly influenced by the malting process since enzyme activity during malting influences the malt extract which limits the accuracy of any NIR prediction based on unmalted barley (Henry, 1985b).

Models for TN prediction were mostly acceptable. The Büchi NIRFlex N-500 instrument delivered dry land whole grain models that were acceptable for screening purposes (r^2 = 0.75; r^2 = 0.76 with variable selection), while irrigation whole grain models were similar, although a much better calibration that could be used with caution in most applications were obtained (r^2 = 0.78; r^2 = 0.85 with variable selection) for irrigation whole grain samples. The Bruker MPA instrument delivered results for dry land (r^2 = 0.64) and irrigation whole grain samples (r^2 = 0.70) that were also acceptable for screening purposes. The Büchi NIRLab N-200 delivered good calibrations for dry land whole grain samples (r^2 = 0.79; r^2 = 0.81 with variable selection) but TN prediction from irrigation whole grain samples was extremely poor. Dry land flour predictions (r^2 = 0.84) were better than those of irrigation flour (r^2 = 0.65). The prediction of nitrogen content from whole grain barley is well established in literature and the results from this study compared well with those of previous reports (r^2 = 0.71, r^2 = 0.83) (Halsey, 1987; Li *et al*., 1995); although some were able to develop excellent calibration models for whole grain barley with r^2 = 0.94 (Edney *et al*., 1994) and r^2 = 0.95 (Sohn *et al*., 2008). Results for ground barley were inferior to what has been reported in literature, with r^2 values as high as 0.99 (Henry, 1985b), and 0.92 (Gill *et al*., 1979; Tragoonrung *et al*., 1990); for development of more effective prediction models for this property, sample ranges should be expanded.

Good results were obtained for TSN prediction from Büchi NIRFlex N-500 dry land whole grain data (r^2 = 0.71; r^2 = 0.73 with variable selection) while an average but acceptable irrigation whole grain model was obtained (r^2 = 0.50). The Bruker MPA instrument delivered poor models in all cases. The Büchi NIRLab N-200 dry land whole grain model (r^2 = 0.55), dry land flour model (r^2 = 0.59; r^2 = 0.61 with variable selection) and irrigation flour model (r^2 = 0.62) were all acceptable for rough screening purposes. These predictions were an improvement on those reported in literature, where a very poor model (r^2 = 0.01) was obtained when TSN was predicted from whole grain barley (Black & Panozzo, 2001).

Models developed for prediction of KI were exceptionally poor and only the Büchi NIRFlex N-500 irrigation whole grain sample model proved to be acceptable for rough screening purposes (r^2 = 0.59). The KI can however be calculated from predicted TN and TSN values, if models for these properties are relatively accurate.

FAN prediction delivered good results for dry land whole grain samples scanned with the Büchi NIRFlex N-500 (r^2 = 0.77) as well as for irrigation whole grain samples (r^2 = 0.63). Bruker MPA calibrations were very poor and not usable for prediction. Büchi NIRLab N-200 dry land whole grain (r^2 = 0.52 with variable selection), irrigation flour (r^2 = 0.54) and dry land flour (r^2 =0.60) models were acceptable for rough screening purposes. Literature reported even poorer results for FAN prediction from whole grain barley (r^2 = 0.10) (Black & Panozzo, 2001) which researchers attributed to the complex nature of this constituent within unmalted barley. The smaller sample range used by these researchers (compared to the range used in this thesis) may also have resulted in poor prediction of this property.

DP prediction from Büchi NIRFlex N-500 dry land whole grain data was acceptable for screening purposes (r^2 = 0.72). The Bruker MPA delivered a good model for dry land whole grain samples (r^2 = 0.59), while acceptable models were only obtained for dry land whole grain (r^2 = 0.56; r^2 = 0.73 with variable selection) and irrigation flour samples (r^2 = 0.58) with Büchi NIRLab N-200 data. Acceptable calibrations (r^2 = 0.59) for predicting DP from whole grain barley were reported in literature. A very poor calibration was also reported (r^2 = 0.39) for DP prediction from whole grain barley (Black & Panozzo, 2001); the small sample range used by these researchers resulted in poor prediction.

Results for prediction of wort viscosity from whole grain barley with Büchi NIRFlex N-500, Bruker MPA and Büchi NIRLab N-200 data were extremely poor and not usable for screening purposes. The use of selected spectral variables showed some improvement in the Büchi NIRLab N-200 dry land flour model (r^2 = 0.65) which is now acceptable for rough screening. These results did not compare well with those in literature, where acceptable calibrations were obtained (r^2 = 0.62, SEP = 0.02 cP) for the prediction of wort viscosity from whole grain unmalted barley (Li *et al.*, 1995) as well as from ground barley (r^2 = 0.65, SEP = 0.60 cP) (Allison *et al.*, 1978). Further research is needed to determine why suitable calibration models could not be developed for this property.

No calibrations accurate enough for rough screening of AAL in dry land or irrigation areas, using either whole grain or ground barley, were calculated. These poor correlations for dry land and irrigation samples can be ascribed to the fact that NIR spectra cannot account for the action of yeast on fermentable sugars during fermentation and can therefore not predict AAL from unmalted barley.

Wort β-glucan prediction was only successful for Büchi NIRFlex N-500 irrigation whole grain samples (r^2 = 0.61) and Büchi NIRLab N-200 irrigation flour with the application of variable

selection (r^2 = 0.54). Unacceptable calibrations for the prediction of wort β-glucan content from whole grain barley have been reported in literature, where an r^2 of 0.25 was obtained (Black & Panozzo, 2001). The inability of the NIR instruments to predict this property is due to the complex nature of the constituent and proteins and starches which are not yet modified by the action of enzymes during malting (Black & Panozzo, 2001). The poor distribution of reference values in the sample range may be the reason for the poor results that were obtained in this study, but further research is needed to conclude why suitable prediction models could not be developed in this study.

Calibration models developed for the combination of samples from two consecutive harvest seasons (2008 and 2009) for the Büchi NIRLab N-200, did not show improved prediction accuracy when compared to the 2008 models alone. For moisture content prediction, the flour models for dry land (r^2 = 0.54) and irrigation (r^2 = 0.60) areas were acceptable for rough screening. TN prediction from dry land whole grain (r^2 = 0.61), irrigation flour (r^2 = 0.60) and dry land flour (r^2 = 0.57) as well as TSN models for dry land whole grain (r^2 = 0.59), irrigation whole grain (r^2 = 0.50 with variable selection) and dry land flour (r^2 = 0.53 with variable selection) were acceptable for rough screening.

The different performances of the three instruments were possibly due to sample presentation differences. The Bruker MPA had a smaller sample cell holder when compared to the larger Petri dishes used for the Büchi instruments which could have contributed to the very poor calibrations obtained with this instrument for most properties, since the area of the sample scanned affects precision of scanning. Flour samples delivered better results than whole grain samples for moisture content, TN, TSN, KI, FAN, wort viscosity and β-glucan content for dry land and irrigation samples. For prediction of DP, irrigation samples resulted in more effective prediction for flour, and dry land samples a better prediction for whole grain. Similar results were obtained for whole grain and flour sample models for plumpness, extract and AAL. In the case of moisture content, TSN, FAN, DP and viscosity, dry land samples delivered better results, while AAL delivered similarly poor results for samples from both the dry land and irrigation areas. In the prediction of β-glucan content, plumpness, extract, TN and KI, better results were obtained for irrigation samples. Variation in sample range distribution was the main reason for differences in prediction accuracy between samples from dry land and irrigation areas.

At this stage the models are acceptable for application as a rough screening technique to separate potentially good malting cultivars from very poor malting cultivars in early stages of the breeding programme. The results were also influenced by bulking of the sample replicates before micro-malting, since the three samples were scanned separately. This resulted in three spectra with an averaged malt quality reference value for all malt properties, which was not representative of the specific sample spectra that were recorded. An accurate prediction can only be attained if the reference values used in calibration development is representative of a single sample and its recorded spectra. Errors of laboratory reference methods (micro-malting) were not obtained in this

study and there is no knowledge on the size of this error. Knowledge of the precision of the reference methods is crucial to the assessment of NIR spectroscopy, as the calibrations developed are dependant on these reference values. Malting is a complex biochemical process which changes the internal structure and components of the barley grain; NIR prediction of malt properties from whole grain barley cannot account for enzyme synthesis and intricate interactions of barley endosperm substrates and enzymes during malting (Henry, 1985b; Li *et al*., 1995; Black & Panozzo, 2001) or the action of yeast during brewing. As a result the technique can only be used as a rough screening method to determine the possible malting behaviour (good or poor) of a line in earlier generations.

Using the first two principal components of PCA biplots, genotype-by-environment (GxE) interactions were interpreted. PCA biplots for the 2008 and 2009 seasons proved that a number of lines had consistent results for a given property over seasons and allowed the identification of lines with poor or good malting quality. However, seasonal differences were also observed where a line had a low value for a property in 2008 but a high value for that same property in 2009, which was attributed to environmental changes over subsequent seasons. Correlation matrices for malt properties confirmed that seasonal differences occurred. This indicates that the GxE interaction should be studied over more than two seasons to determine if breeding lines can deliver consistent results for a given locality or under certain growing conditions (dry land or irrigation) for more than one harvest season. Elite trials lasting three seasons could be sufficient for assessment of seasonal and locality effects on breeding lines. Irrigation environments delivered more consistent quality for several properties over locations and seasons, due to the more controlled environmental conditions. These PCA biplots can be used as an additional tool to allow breeders to make conclusions regarding the quality of specific breeding lines over a series of localities as well as growing seasons. For malting quality evaluations, field replicates should ideally not be bulked, as bulking ignores the variation present within a single field trial.

NIR calibrations suitable for rough screening of several malting quality properties were calculated, and the usefulness of PCA biplots for the study of genetic and environmental effects on these properties was established. Further development of the work presented in this thesis requires samples from at least another harvest and the determination of malting properties from individual samples.

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Appendices

Appendix 1

Table 1 Summary of calibration and validation results for test set validation models from the Büchi NIRFlex N-500 data (The Unscrambler software) for dry land whole grain samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture (%)	None	0.66	0.48	0.69	0.48	-0.08	1.36	8
	Mean norm	0.68	0.52	0.70	0.46	-0.09	1.35	5
	SNV	0.66	0.53	0.69	0.47	-0.13	1.36	6
	1st der	0.63	0.58	0.75	0.39	-0.05	1.26	5
	2nd der	0.69	0.50	0.76	0.36	-0.12	1.24	4
	1st der & SNV	0.69	0.49	0.76	0.36	-0.09	1.25	4
	2nd der & SNV	0.71	0.50	0.79	0.31	-0.09	1.19	2
Plumpness (%)	None	2.67	0.51	3.04	0.36	0.20	1.24	12
	Mean norm	2.63	0.52	3.03	0.37	0.13	1.25	12
	SNV	2.72	0.49	3.05	0.36	0.17	1.24	11
	1st der	2.77	0.47	3.15	0.32	0.13	1.20	6
	2nd der	3.07	0.35	3.24	0.35	0.07	1.16	3
	1st der & SNV	2.75	0.48	3.13	0.33	0.18	1.21	8
	2nd der & SNV	2.93	0.41	3.29	0.25	0.19	1.15	2
Extract (%)	None	0.63	0.70	0.79	0.39	0.1350	1.23	14
	Mean norm	0.67	0.65	0.79	0.38	0.1480	1.22	12
	SNV	0.72	0.60	0.80	0.36	0.1670	1.21	9
	1st der	0.69	0.63	0.79	0.36	0.1220	1.23	9
	2nd der	0.70	0.62	0.78	0.37	0.1760	1.25	8
	1st der & SNV	0.69	0.63	0.78	0.38	0.1513	1.24	8
	2nd der & SNV	0.75	0.56	0.77	0.39	0.1930	1.26	8
TN (%)	None	0.12	0.79	0.11	0.73	0.0130	1.92	9
	Mean norm	0.11	0.83	0.11	0.73	0.0150	1.92	9
	SNV	0.10	0.84	0.11	0.75	0.0010	2.01	10
	1st der	0.09	0.87	0.11	0.75	-0.0044	1.99	10
	2nd der	0.10	0.84	0.11	0.73	-0.0096	1.90	8
	1st der & SNV	0.09	0.86	0.11	0.75	-0.0084	2.01	9
	2nd der & SNV	0.10	0.83	0.11	0.76	-0.0055	2.05	7
TSN (%)	None	0.06	0.76	0.07	0.66	0.0060	1.72	12
	Mean norm	0.06	0.53	0.06	0.46	0.0049	1.81	9
	SNV	0.05	0.80	0.07	0.69	0.0039	1.78	13
	1st der	0.06	0.74	0.06	0.69	0.0049	1.81	9
	2nd der	0.06	0.76	0.07	0.67	-0.0007	1.75	8
	1st der & SNV	0.06	0.75	0.06	0.71	-0.0008	1.84	8
	2nd der & SNV	0.06	0.71	0.07	0.68	0.0047	1.76	8
KI	None	3.22	0.08	4.37	0.11	0.4760	1.05	1
	Mean norm	2.48	0.46	4.07	0.46	0.1920	1.13	15
	SNV	2.49	0.45	4.06	0.22	0.1740	1.13	14
	1st der	2.48	0.33	4.07	0.22	0.1920	1.13	15
	2nd der	2.61	0.40	4.19	0.17	0.2290	1.10	9
	1st der & SNV	2.63	0.39	4.02	0.24	0.1378	1.15	7
	2nd der & SNV	2.78	0.32	4.11	0.20	0.3130	1.12	8
FAN (mg/L)	None	22.26	0.64	27.82	0.61	4.9020	1.59	12
	Mean norm	18.68	0.76	26.70	0.64	3.8010	1.66	16
	SNV	18.05	0.75	28.37	0.77	3.3580	1.56	16
	1st der	22.50	0.64	26.36	0.65	4.7800	1.68	8
	2nd der	24.42	0.58	27.88	0.61	5.0570	1.59	7
	1st der & SNV	22.16	0.66	26.39	0.65	3.7600	1.68	8
	2nd der & SNV	23.77	0.61	26.64	0.65	4.8225	1.66	8

Table 1 continued

DP (W.K.)	None	55.82	0.76	62.45	0.69	-12.789	1.80	13
	Mean norm	56.95	0.74	60.35	0.71	-14.038	1.87	12
	SNV	58.62	0.73	59.42	0.72	-13.108	1.89	11
	1st der	68.18	0.63	66.52	0.63	-7.957	1.69	6
	2nd der	68.45	0.63	64.41	0.63	-8.427	1.75	5
	1st der & SNV	59.95	0.72	64.80	0.67	-18.722	1.74	7
	2nd der & SNV	65.55	0.66	62.41	0.15	-16.684	1.80	7
Viscosity (cP)	None	0.02	0.41	0.03	0.10	-0.0071	1.21	7
	Mean norm	0.01	0.66	0.02	0.001	-0.0055	1.32	15
	SNV	0.02	0.40	0.03	0.10	-0.0072	1.21	5
	1st der	0.01	0.65	0.02	0.25	-0.0069	1.32	11
	2nd der	0.01	0.62	0.02	0.26	-0.0073	1.38	10
	1st der & SNV	0.02	0.48	0.02	0.21	-0.0085	1.32	6
	2nd der & SNV	0.02	0.42	0.02	0.17	-0.0079	1.28	6
AAL	None	0.36	0.36	1.80	0.15	0.2220	1.06	5
	Mean norm	0.39	0.39	1.73	0.20	0.2190	1.11	7
	SNV	0.35	0.35	1.75	0.18	0.2380	1.10	4
	1st der	0.35	0.35	1.80	0.15	0.1410	1.06	2
	2nd der	0.36	0.36	1.82	0.15	0.1690	1.05	3
	1st der & SNV	1.79	0.32	1.80	0.15	0.1830	1.06	1
	2nd der & SNV	1.79	0.31	1.81	0.14	0.1770	1.06	1
β-glucans (mg/L)	None	23.87	0.56	56.09	0.45	-24.7170	1.28	16
	Mean norm	25.18	0.51	56.89	0.42	-24.3410	1.26	14
	SNV	26.00	0.48	58.76	0.38	-22.8990	1.22	12
	1st der	25.12	0.51	56.52	0.43	-25.7027	1.27	10
	2nd der	22.32	0.61	55.19	0.46	-24.3761	1.30	11
	1st der & SNV	15.10	0.15	24.77	0.09	-11.5930	2.89	4
	2nd der & SNV	15.28	0.13	24.78	0.09	-11.5830	2.89	5

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 2 Calibration and validation results for uncertainty tested models from the Büchi NIRFlex N-500 data (The Unscrambler software) for dry land whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	None	0.70	0.47	0.74		0.41	0.0014	1.27	6
	Test set	None	0.70	0.49		0.71	0.43	-0.1349	1.33	6
	CV	SNV	0.74	0.41	0.75		0.38	-0.0016	1.25	2
	Test set	SNV	0.73	0.44		0.76	0.36	-0.1152	1.24	2
Plumpness	CV	none	2.89	0.42	2.95		0.39	-0.000002	1.28	8
(%)	Test set	none	3.03	0.37		3.23	0.28	0.2113	1.17	8
	CV	SNV	2.83	0.44	2.94		0.40	-0.0027	1.28	9
	Test set	SNV	2.77	0.47		3.03	0.36	0.2008	1.24	3
Extract (%)	CV	none	0.70	0.58	0.76		0.51	0.0021	1.27	9
	Test set	none	0.74	0.57		0.75	0.40	0.1180	1.30	9
	CV	SNV	0.74	0.53	0.81		0.44	0.0028	1.19	8
	Test set	SNV	0.78	0.52		0.77	0.36	0.0975	1.26	8
TN (%)	CV	SNV	0.11	0.81	0.11		0.78	-0.0002	1.91	7
	Test set	SNV	0.11	0.82		0.11	0.76	0.0061	1.97	7
	CV	1st der	0.12	0.76	0.12		0.74	-0.0002	1.75	4
	Test set	1st der	0.12	0.78		0.12	0.69	-0.0095	1.81	4
TSN (%)	CV	none	0.06	0.69	0.07		0.64	-0.0003	1.68	8
	Test set	none	0.07	0.68		0.07	0.67	0.0032	1.74	8
	CV	1st der	0.06	0.71	0.07		0.66	0.0001	1.72	7
	Test set	1st der	0.06	0.70		0.06	0.73	0.0010	1.90	6
KI	CV	none	3.65	0.09	3.71		0.06	-0.0037	1.24	2
	Test set	none	3.25	0.07		4.37	0.11	0.5090	1.05	1
	CV	SNV	3.60	0.11	3.68		0.07	-0.0065	1.25	3
	Test set	SNV	3.19	0.10		4.42	0.08	0.3636	1.04	3
FAN (mg/L)	CV	SNV	24.07	0.64	26.03		0.64	-0.0290	1.70	9
	Test set	SNV	24.23	0.59		25.84	0.68	5.5080	1.71	8
	CV	1st der	24.95	0.62	26.53		0.62	-0.0218	1.67	6
	Test set	1st der	26.02	0.53		26.27	0.67	6.0457	1.68	4
DP (W.K.)	CV	none	62.11	0.70	66.75		0.65	-0.034	1.69	10
	Test set	none	63.91	0.68		59.70	0.72	-8.788	1.89	9
	CV	SNV	67.83	0.64	70.64		0.61	0.038	1.59	6
	Test set	SNV	70.04	0.61		65.72	0.66	-3.411	1.71	5
Viscosity (cP)	CV	none	0.02	0.27	0.02		0.19	0.00000006	1.38	6
	Test set	none	0.02	0.19		0.03	0.05	-0.0063	1.21	2
	CV	2nd der	0.02	0.33	0.02		0.28	-0.00002	1.51	4
	Test set	2nd der	0.02	0.34		0.02	0.26	-0.0078	1.38	3
AAL	CV	none	1.70	0.34	1.78		0.28	0.0023	1.07	6
	Test set	none	1.89	0.24		1.75	0.18	0.1720	1.09	3
	CV	mean norm	1.72	0.33	1.78		0.28	-0.0044	1.08	4
	Test set	mean norm	1.78	0.33		1.72	0.20	0.2630	1.11	3
β-glucans	CV	none	15.86	0.06	16.06		0.04	-0.0002	4.46	1
(mg/L)	Test set	none	15.86	0.06		24.75	0.13	-12.5968	2.90	1
	CV	2nd der	20.75	0.02	20.38		0.002	-0.0146	3.52	1
	Test set	2nd der	16.28	0.01		25.62	0.05	-12.927	2.80	1

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 3 Summary of calibration and test set validation results for models from the Büchi NIRFlex N-500 data (The Unscrambler software) for irrigation whole grain samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture (%)	None	0.58	0.21	0.60	0.18	-0.0126	1.10	5
	Mean norm	0.60	0.16	0.60	0.18	0.0031	1.10	4
	SNV	0.60	0.17	0.60	0.18	0.0032	1.10	3
	1st der	0.55	0.29	0.60	0.19	-0.0538	1.10	4
	2nd der	0.55	0.30	0.60	0.18	-0.0248	1.09	4
	1st der& SNV	0.57	0.25	0.62	0.14	0.0212	1.07	3
	2nd der & SNV	0.56	0.28	0.61	0.16	0.0144	1.09	3
Plumpness	None	3.69	0.04	3.49	0.05	-0.6610	1.29	3
(%)	Mean norm	3.70	0.03	3.47	0.07	-0.0960	1.29	2
	SNV	3.71	0.02	3.50	0.05	-0.0980	1.28	1
	1st der	3.67	0.05	3.49	0.05	-0.0576	1.29	2
	2nd der	3.66	0.05	3.49	0.05	-0.1007	1.29	3
	1st der & SNV	3.69	0.03	3.50	0.05	-0.0964	1.28	1
	2nd der & SNV	3.66	0.05	3.51	0.04	-0.1316	1.28	2
Extract (%)	None	0.57	0.66	0.93	0.53	0.1620	1.46	10
	Mean norm	0.62	0.61	0.95	0.52	0.1620	1.44	7
	SNV	0.63	0.60	0.96	0.51	0.1660	1.42	7
	1st der	0.60	0.63	0.88	0.60	0.0975	1.54	5
	2nd der	0.66	0.56	0.91	0.59	0.0438	1.50	4
	1st der & SNV	0.55	0.70	0.90	0.57	0.1183	1.51	7
	2nd der & SNV	0.64	0.59	0.95	0.55	0.1460	1.44	5
TN (%)	None	0.08	0.68	0.10	0.77	-0.0466	1.96	9
	Mean norm	0.08	0.70	0.11	0.74	-0.0367	1.85	10
	SNV	0.09	0.61	0.11	0.74	-0.0603	1.85	7
	1st der	0.09	0.60	0.10	0.78	-0.0530	1.88	6
	2nd der	0.07	0.76	0.11	0.69	-0.0350	1.79	9
	1st der & SNV	0.07	0.75	0.11	0.72	-0.0310	1.86	9
	2nd der & SNV	0.08	0.74	0.11	0.69	-0.0350	1.78	9
TSN (%)	None	0.05	0.72	0.09	0.44	-0.0369	1.34	12
	Mean norm	0.06	0.66	0.08	0.53	-0.0412	1.46	10
	SNV	0.06	0.45	0.08	0.50	-0.0393	1.39	6
	1st der	0.07	0.52	-0.03	0.41	-0.0298	-3.89	5
	2nd der	0.06	0.65	0.09	0.40	-0.0212	1.26	7
	1st der & SNV	0.05	0.71	0.08	0.48	-0.0330	1.39	8
	2nd der & SNV	0.06	0.65	0.09	0.38	-0.0232	1.26	7
KI	None	2.14	0.72	3.44	0.47	-0.9079	1.36	12
	Mean norm	2.88	0.50	3.66	0.39	-0.4120	1.28	7
	SNV	2.21	0.71	3.40	0.48	-0.8790	1.38	10
	1st der	2.45	0.64	3.49	0.46	-0.5114	1.34	7
	2nd der	2.85	0.51	3.74	0.40	0.4260	1.25	5
	1st der & SNV	2.52	0.62	3.56	0.43	-0.4120	1.32	5
	2nd der & SNV	3.17	0.39	3.68	0.38	0.0440	1.27	3
FAN (mg/L)	None	19.43	0.59	29.05	0.63	-11.7890	1.54	10
	Mean norm	21.12	0.52	25.70	0.49	-3.4430	1.75	7
	SNV	25.34	0.51	24.56	0.53	-2.4649	1.83	7
	1st der	21.80	0.52	24.87	0.52	3.4378	1.80	5
	2nd der	23.74	0.40	29.14	0.34	4.7586	1.54	4
	1st der & SNV	17.74	0.66	28.02	0.40	-0.1990	1.60	7
	2nd der & SNV	22.76	0.44	29.33	0.34	3.6590	1.53	4

Table 3 continued

DP (W.K.)	None	41.04	0.71	91.37	0.28	1.9080	1.17	12
	Mean norm	42.49	0.69	93.19	0.26	2.8840	1.15	11
	SNV	40.95	0.71	89.71	0.31	6.6840	1.19	11
	1^{st} der	43.18	0.68	85.39	0.36	10.6320	1.25	8
	2^{nd} der	55.41	0.47	86.63	0.36	20.7850	1.23	5
	1^{st} der & SNV	51.20	0.54	84.30	0.40	18.2450	1.27	6
	2^{nd} der & SNV	53.22	0.51	86.95	0.35	16.8730	1.23	5
Viscosity (cP)	None	0.02	0.51	0.02	0.31	-0.0107	1.57	13
	Mean norm	0.02	0.51	0.02	0.37	-0.0102	1.64	10
	SNV	0.01	0.53	0.02	0.36	-0.0099	1.62	10
	1^{st} der	0.01	0.62	0.02	0.40	-0.0087	1.67	10
	2^{nd} der	0.01	0.61	0.02	0.29	-0.0094	1.55	9
	1^{st} der & SNV	0.01	0.56	0.02	0.37	-0.0093	1.61	8
	2^{nd} der & SNV	0.01	0.64	0.02	0.25	-0.0095	1.50	9
AAL	None	2.10	0.28	1.73	0.22	-0.2330	1.10	4
	Mean norm	2.13	0.26	1.83	0.14	-0.0450	1.04	4
	SNV	2.12	0.25	1.82	0.15	-0.0490	1.04	3
	1^{st} der	2.15	0.24	1.71	0.20	-0.1860	1.11	2
	2^{nd} der	2.15	0.24	1.85	0.09	0.0164	1.03	2
	1^{st} der & SNV	2.23	0.18	1.82	0.10	-0.2350	1.05	1
	2^{nd} der & SNV	2.06	0.30	1.82	0.13	0.0665	1.04	2
β-glucans	None	27.56	0.55	32.74	0.54	3.1336	3.45	9
(mg/L)	Mean norm	27.86	0.53	33.56	0.52	3.5519	3.37	9
	SNV	28.11	0.53	33.42	0.52	3.2250	3.38	8
	1^{st} der	25.39	0.61	30.26	0.60	-3.8200	3.73	7
	2^{nd} der	25.99	0.60	30.31	0.59	-2.9750	3.73	6
	1^{st} der & SNV	26.73	0.57	29.83	0.61	-5.2311	3.79	6
	2^{nd} der & SNV	28.24	0.63	29.51	0.53	-4.0960	3.83	5

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1^{st} der=Savitzky-Golay first derivative, 9 points; 2^{nd} der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 4 Calibration and validation results for uncertainty tested models from the Büchi NIRFlex N-500 data (The Unscrambler software) for irrigation whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.60	0.16	0.63		0.08	0.0056	1.04	3
	Test set	none	0.61	0.13		0.62	0.12	-0.0317	1.06	2
	CV	1st der	0.61	0.13	0.65		0.04	0.0108	1.01	2
	Test set	1st der	0.62	0.10		0.68	0.005	0.0130	0.97	2
Plumpness (%)	CV	None	3.63	0.04	3.65		0.03	0.0023	1.23	2
	Test set	None	3.69	0.04		3.51	0.04	-0.0577	1.28	2
	CV	mean norm	3.64	0.03	3.66		0.02	0.0020	1.23	1
	Test set	mean norm	3.62	0.03		3.47	0.07	-0.0962	1.29	2
Extract (%)	CV	1st der	0.70	0.61	0.78		0.53	0.00004	1.75	4
	Test set	1st der	0.66	0.56		0.81	0.69	0.0237	1.67	4
	CV	2nd der	0.70	0.62	0.77		0.54	0.0041	1.77	4
	Test set	2nd der	0.67	0.55		0.80	0.69	-0.0065	1.70	4
TN (%)	CV	None	0.09	0.73	0.10		0.66	-0.0005	1.97	8
	Test set	None	0.09	0.61		0.10	0.80	-0.0450	2.02	7
	CV	1st der	0.09	0.72	0.10		0.67	-0.0016	1.99	5
	Test set	1st der	0.09	0.60		0.09	0.85	-0.0457	2.13	5
TSN (%)	CV	None	0.07	0.61	0.07		0.51	-0.0002	1.56	8
	Test set	None	0.06	0.59		0.08	0.47	-0.0230	1.38	7
	CV	Mean norm	0.07	0.50	0.08		0.40	0.0008	1.42	6
	Test set	Mean norm	0.09	0.12		0.10	0.44	-0.0418	1.22	3
KI	CV	None	2.63	0.62	2.97		0.52	0.0280	1.58	8
	Test set	None	2.53	0.61		3.02	0.59	-0.0819	1.50	8
	CV	SNV	2.74	0.59	3.15		0.47	0.0226	1.48	7
	Test set	SNV	2.86	0.50		3.25	0.54	0.4698	1.44	7
FAN (mg/L)	CV	none	21.28	0.56	23.33		0.47	-0.0511	1.92	7
	Test set	none	20.85	0.53		23.63	0.57	-5.9440	1.90	7
	CV	SNV	20.30	0.60	22.13		0.53	-0.0247	2.03	6
	Test set	SNV	19.79	0.58		21.97	0.63	-6.3984	2.04	6
DP (W.K.)	CV	none	62.38	0.50	68.91		0.39	0.2750	1.55	8
	Test set	none	62.56	0.32		85.95	0.40	28.1070	1.24	6
	CV	1st der	76.66	0.24	80.01		0.18	-0.3320	1.34	3
	Test set	1st der	65.24	0.26		94.94	0.22	25.7155	1.13	3
Viscosity (cP)	CV	none	0.02	0.13	0.02		0.09	-0.0001	1.57	1
	Test set	none	0.02	0.18		0.03	0.07	-0.0092	1.23	1
	CV	1st der	0.02	0.40	0.02		0.21	-0.0005	1.67	5
	Test set	2nd der	0.02	0.07		0.03	0.05	-0.0138	1.34	1
AAL	CV	none	1.71	0.46	1.89		0.35	-0.0054	1.01	8
	Test set	none	2.21	0.23		1.70	0.22	-0.3878	1.12	4
	CV	1st der	1.81	0.37	1.91		0.30	0.0107	0.99	4
	Test set	1st der	2.15	0.24		1.71	0.19	-0.1840	1.11	2
β-glucans	CV	none	29.28	0.54	31.96		0.45	-1.0950	3.54	7
(mg/L)	Test set	none	36.62	0.20		42.90	0.22	-0.4980	2.63	4
	CV	1st der	27.72	0.58	31.35		0.47	-0.6030	3.60	5
	Test set	1st der	30.27	0.45		38.58	0.36	-2.5600	2.93	4

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 5 Summary of calibration and test set validation results for models from the Bruker MPA data (OPUS software) for dry land whole grain samples

Property	Pretreatment	Calibration		Validation				
		RMSEE	R^2	RMSEP	r^2	Bias	RPD	PLS
Moisture (%)	No spectral pretreatment	0.68	0.53	0.65	0.50	0.0337	1.41	6
	Min max normalization	0.66	0.56	0.63	0.53	0.0771	1.46	6
	SNV	0.70	0.50	0.67	0.47	0.0226	1.37	4
	1st der	0.51	0.74	0.83	0.23	-0.0273	1.11	6
	2nd der	0.94	0.08	0.91	0.03	0.0016	1.01	1
	1st der & SNV	0.45	0.79	0.81	0.25	-0.0895	1.14	6
	1st der & MSC	0.47	0.78	0.81	0.26	-0.0657	1.14	6
Plumpness (%)	No spectral pretreatment	2.93	0.39	2.76	0.29	0.0818	1.18	13
	Min max normalization	3.01	0.36	2.90	0.23	0.0913	1.12	12
	SNV	2.94	0.39	2.87	0.25	0.106	1.14	12
	1st der	3.04	0.34	2.99	0.20	0.129	1.09	9
	2nd der	3.56	0.10	3.13	0.09	0.373	1.05	3
	1st der & SNV	3.24	0.25	3.15	0.11	0.333	1.04	6
	1st der & MSC	3.24	0.25	3.16	0.10	0.33	1.04	6
Extract (%)	No spectral pretreatment	0.92	0.35	0.85	0.24	-0.0788	1.14	5
	Min max normalization	0.89	0.36	0.90	0.14	-0.0102	1.07	4
	SNV	0.94	0.29	0.87	0.20	-0.0472	1.11	3
	1st der	0.82	0.49	0.88	0.22	-0.0472	1.09	6
	2nd der	0.96	0.29	0.95	0.12	-0.159	1.03	4
	1st der & SNV	0.76	0.57	0.90	0.20	-0.0412	1.08	6
	1st der & MSC	0.76	0.56	0.89	0.21	-0.0378	1.08	6
TN (%)	No spectral pretreatment	0.08	0.90	0.14	0.58	-0.0147	1.51	15
	Min max normalization	0.09	0.90	0.13	0.64	-0.0086	1.65	13
	SNV	0.09	0.88	0.13	0.64	-0.0146	1.66	12
	1st der	0.13	0.75	0.16	0.49	-0.0049	1.36	7
	2nd der	0.12	0.80	0.19	0.37	0.0217	1.17	7
	1st der & SNV	0.13	0.74	0.17	0.43	-0.0049	1.28	6
	1st der & MSC	0.13	0.74	0.17	0.42	-0.0032	1.28	6
TSN (%)	No spectral pretreatment	0.05	0.81	0.09	0.44	-0.0086	1.32	13
	Min max normalization	0.05	0.84	0.09	0.42	-0.0108	1.3	14
	SNV	0.05	0.81	0.09	0.43	-0.0065	1.31	14
	1st der	0.08	0.57	0.09	0.37	-0.0062	1.26	6
	2nd der	0.08	0.59	0.10	0.24	0.0017	1.13	6
	1st der & SNV	0.08	0.51	0.10	0.28	-0.0057	1.17	5
	1st der & MSC	0.07	0.61	0.10	0.27	-0.0109	1.16	6
KI	No spectral pretreatment	3.21	0.10	4.53	0.03	-0.485	1.02	4
	Min max normalization	3.22	0.09	4.57	0.02	-0.500	1.01	2
	SNV	3.21	0.09	4.56	0.02	-0.482	1.01	2
	1st der	2.74	0.30	4.49	0.08	-0.721	1.03	5
	2nd der	2.75	0.28	4.52	0.07	-0.774	1.03	4
	1st der & SNV	2.46	0.42	4.47	0.09	-0.585	1.03	6
	1st der & MSC	2.76	0.26	4.43	0.09	-0.628	1.05	4
FAN (mg/L)	No spectral pretreatment	21.80	0.69	34.80	0.39	-6.170	1.28	14
	Min max normalization	20.90	0.71	35.20	0.39	-7.670	1.28	14
	SNV	24.00	0.61	36.80	0.35	-9.430	1.24	11
	1st der	24.40	0.59	36.80	0.34	-8.960	1.23	8
	2nd der	26.90	0.50	38.00	0.29	-8.530	1.19	6
	1st der & SNV	28.60	0.42	37.80	0.31	-9.650	1.20	5
	1st der & MSC	28.60	0.42	37.90	0.30	-9.390	1.20	5

Table 5 continued

DP (W.K.)	No spectral pretreatment	53.80	0.78	74.30	0.59	9.800	1.53	13
	Min max normalization	68.30	0.65	79.30	0.51	4.680	1.43	9
	SNV	63.80	0.69	79.90	0.51	-4.780	1.42	9
	1st der	53.80	0.78	75.20	0.54	9.040	1.47	10
	2nd der	70.20	0.62	91.70	0.36	13.700	1.25	7
	1st der & SNV	55.40	0.77	85.70	0.45	12.700	1.33	9
	1st der & MSC	55.80	0.76	85.60	0.45	14.400	1.34	9
Viscosity (cP)	No spectral pretreatment	0.02	0.30	0.03	0.10	0.006	1.04	6
	Min max normalization	0.02	0.25	0.03	0.09	0.006	1.05	4
	SNV	0.02	0.23	0.02	0.12	0.007	1.07	3
	1st der	0.02	0.22	0.03	0.05	0.007	0.99	3
	2nd der	0.02	0.13	0.03	0.004	0.009	0.96	2
	1st der & SNV	0.02	0.33	0.03	0.05	0.008	1.00	3
	1st der & MSC	0.02	0.34	0.03	0.05	0.008	1.00	3
AAL	No spectral pretreatment	2.03	0.05	1.73	0.11	-0.345	1.06	1
	Min max normalization	1.81	0.25	1.67	0.17	-0.237	1.09	2
	SNV	1.77	0.29	1.69	0.16	-0.209	1.07	2
	1st der	1.94	0.14	1.71	0.13	-0.332	1.07	1
	2nd der	1.86	0.21	1.77	0.11	-0.382	1.03	2
	1st der & SNV	1.93	0.15	1.76	0.10	1.040	1.04	1
	1st der & MSC	1.93	0.15	1.76	0.10	-0.367	1.04	1
β-glucans	No spectral pretreatment	16.20	0.06	19.50	0.02	5.830	1.00	1
(mg/L)	Min max normalization	16.50	0.04	19.30	0.03	5.570	1.01	2
	SNV	16.60	0.02	19.10	0.07	5.680	1.02	1
	1st der	16.50	0.03	20.60	0.06	6.400	0.95	1
	2nd der	16.50	0.02	20.30	0.04	6.280	0.97	1
	1st der & SNV	16.50	0.03	20.60	0.06	6.390	0.96	1
	1st der & MSC	16.50	0.03	20.60	0.06	6.380	0.96	1

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC=standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; MSC=multiplicative scatter correction; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; Normal=min max normalization; none=no spectral pre-treatment

Table 6 Summary of calibration and test set validation results for models from the Bruker MPA data (OPUS software) for irrigation whole grain samples

Property	Pretreatment	Calibration		Validation				
		RMSEE	R^2	RMSEP	r^2	Bias	RPD	PLS
Moisture (%)	None	0.59	0.27	0.60	0.14	0.0168	1.03	5
	Normal	0.59	0.22	0.59	0.12	0.0282	1.05	4
	SNV	0.55	0.29	0.58	0.16	0.0011	1.07	3
	1st der	0.63	0.06	0.59	0.10	-0.0326	1.05	1
	2nd der	0.57	0.12	0.60	0.12	-0.0693	1.05	2
	1st der & SNV	0.63	0.06	0.59	0.10	-0.0326	1.05	1
	1st der & MSC	0.63	0.06	0.59	0.10	-0.0321	1.05	1
Plumpness (%)	None	2.08	0.60	2.31	0.49	-0.1730	1.38	7
	Normal	2.01	0.63	2.43	0.43	-0.1960	0.31	15
	SNV	2.02	0.63	2.36	0.46	-0.2350	1.35	14
	1st der	1.68	0.74	2.65	0.37	-0.1160	1.20	15
	2nd der	2.95	0.18	2.98	0.13	-0.0591	1.06	4
	1st der & SNV	2.37	0.48	2.79	0.26	-0.0998	1.14	7
	1st der & MSC	1.68	0.74	2.82	0.31	0.0198	1.13	15
Extract (%)	None	0.67	0.56	1.14	0.31	-0.2070	1.20	9
	Normal	0.58	0.68	0.94	0.55	-0.0836	1.45	10
	SNV	0.57	0.69	0.93	0.55	-0.0698	1.45	10
	1st der	0.69	0.54	1.23	0.18	-0.1550	1.11	6
	2nd der	0.92	0.14	1.27	0.15	-0.1710	1.08	2
	1st der & SNV	0.57	0.69	1.20	0.22	-0.0856	1.13	7
	1st der & MSC	0.57	0.68	1.20	0.21	-0.0867	1.12	7
TN (%)	None	0.07	0.78	0.13	0.63	0.0512	1.63	12
	Normal	0.07	0.80	0.12	0.67	0.0509	1.74	12
	SNV	0.07	0.79	0.12	0.70	0.0550	1.79	12
	1st der	0.05	0.90	0.15	0.47	0.0555	1.37	11
	2nd der	0.10	0.58	0.18	0.29	0.0831	1.19	6
	1st der & SNV	0.05	0.89	0.15	0.46	0.0561	1.36	11
	1st der & MSC	0.06	0.86	0.15	0.48	0.0593	1.38	10
TSN (%)	None	0.05	0.73	0.11	0.21	0.0252	1.09	12
	Normal	0.05	0.69	0.10	0.30	0.0298	1.18	10
	SNV	0.05	0.70	0.10	0.30	0.0302	1.20	10
	1st der	0.08	0.16	0.12	0.08	0.0475	1.04	2
	2nd der	0.08	0.15	0.12	0.06	0.0502	1.03	2
	1st der & SNV	0.08	0.17	0.12	0.09	0.0461	1.05	2
	1st der & MSC	0.08	0.17	0.12	0.09	0.0461	1.05	2
KI	None	3.84	0.13	4.08	0.24	0.0885	1.14	3
	Normal	3.05	0.47	4.05	0.27	0.5160	1.16	8
	SNV	3.20	0.41	4.08	0.26	0.6960	1.16	7
	1st der	3.70	0.18	4.33	0.14	0.3250	1.08	2
	2nd der	3.71	0.18	4.46	0.09	0.4060	1.05	2
	1st der & SNV	3.74	0.16	4.39	0.11	0.2950	1.06	2
	1st der & MSC	3.74	0.16	1.39	0.11	0.2980	1.06	2
FAN (mg/L)	None	16.40	0.72	36.80	0.33	4.2200	1.22	12
	Normal	17.10	0.69	35.60	0.37	4.7600	1.26	10
	SNV	17.20	0.69	35.30	0.38	4.2100	1.27	10
	1st der	28.00	0.13	42.80	0.13	9.4200	1.07	2
	2nd der	27.80	0.15	43.80	0.09	10.2000	1.05	2
	1st der & SNV	27.90	0.14	42.70	0.13	9.1000	1.07	2
	1st der & MSC	27.90	0.14	42.70	0.13	9.1200	1.07	2

Table 6 continued

DP (W.K.)	None	46.70	0.65	96.20	0.21	-15.1000	1.12	12
	Normal	42.70	0.71	94.70	0.22	-7.5400	1.13	11
	SNV	48.00	0.63	98.20	0.19	-18.9000	1.10	10
	1st der	37.80	0.77	100.00	0.20	-27.3000	1.10	9
	2nd der	24.30	0.91	104.00	0.20	-29.6000	1.07	12
	1st der & SNV	41.10	0.72	99.60	0.20	-26.9000	1.11	8
	1st der & MSC	41.20	0.72	99.70	0.20	-27.0000	1.11	8
Viscosity (cP)	None	0.02	0.27	0.03	0.14	0.0097	1.08	4
	Normal	0.02	0.30	0.03	0.09	0.0103	1.04	5
	SNV	0.02	0.26	0.03	0.086	0.0104	1.03	3
	1st der	0.02	0.04	0.03	0.003	0.0120	1.00	1
	2nd der	0.02	0.06	0.03	0.0002	0.0119	0.99	1
	1st der & SNV	0.02	0.04	0.03	0.003	0.0120	1.00	1
	1st der & MSC	0.02	0.04	0.03	0.003	0.0120	1.00	1
AAL	None	2.44	0.07	1.99	0.0123	0.3950	0.97	1
	Normal	2.35	0.15	1.96	0.0537	0.2190	0.97	3
	SNV	2.40	0.10	1.97	0.0332	0.1730	0.97	1
	1st der	2.39	0.11	2.14	0.0004	0.4340	0.90	1
	2nd der	2.49	0.03	1.97	0.0001	0.2580	0.97	1
	1st der & SNV	2.44	0.07	2.08	0.0001	0.4810	0.93	1
	1st der & MSC	2.44	0.07	2.08	0.0001	0.4800	0.93	1
β-glucans (mg/L)	None	27.30	0.64	39.60	0.35	-6.2500	1.21	11
	Normal	27.70	0.63	40.40	0.32	-0.8270	1.17	10
	SNV	25.50	0.69	38.50	0.37	-2.6300	1.23	10
	1st der	39.40	0.21	37.70	0.42	-1.3900	1.26	3
	2nd der	32.60	0.47	39.90	0.29	-0.8920	1.19	5
	1st der & SNV	39.30	0.22	37.40	0.43	-1.4100	1.27	3
	1st der & MSC	39.30	0.22	37.50	0.43	-1.4000	1.27	3

TN=total nitrogen; TSN=total soluble nitrogen; KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); RMSEE=root mean square error of estimation; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least square factors; MSC=multiplicative scatter correction; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative,17 points; Normal=min max normalization; none=no spectral pre-treatment

Table 7 Summary of calibration and test set validation results for models from the Büchi NIRLab N-200 data for dry land whole grain samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r²	Bias	RPD	PLS
Moisture (%)	None	0.61	0.47	0.71	0.47	0.0081	1.30	4
	Mean norm	0.68	0.50	0.72	0.41	0.0420	1.30	3
	SNV	0.70	0.46	0.71	0.43	0.0798	1.31	2
	1st der	0.72	0.44	0.72	0.41	0.0592	1.29	2
	2nd der	0.71	0.46	0.69	0.45	0.0021	1.34	3
	1st der & SNV	0.78	0.36	0.71	0.45	0.0709	1.31	1
	2nd der & SNV	0.73	0.43	0.69	0.47	0.0514	1.36	2
Plumpness (%)	None	3.04	0.30	3.37	0.27	0.3433	1.20	5
	Mean norm	2.65	0.45	3.48	0.25	0.5291	1.16	6
	SNV	3.06	0.34	3.46	0.24	0.3363	1.17	5
	1st der	3.34	0.23	3.52	0.20	0.0192	1.15	2
	2nd der	3.16	0.31	3.37	0.27	-0.0245	1.20	2
	1st der & SNV	3.07	0.35	3.51	0.22	0.2441	1.15	3
	2nd der & SNV	1.96	0.71	3.18	0.36	0.5059	1.27	8
Extract (%)	None	0.91	0.44	0.79	0.44	0.0867	1.34	5
	Mean norm	0.95	0.40	0.78	0.46	0.1542	1.36	4
	SNV	0.72	0.66	0.76	0.50	0.1498	1.39	9
	1st der	0.86	0.50	0.74	0.53	0.1052	1.43	4
	2nd der	0.79	0.58	0.73	0.56	0.0869	1.46	5
	1st der & SNV	0.83	0.52	0.75	0.50	0.0742	1.41	5
	2nd der & SNV	0.73	0.64	0.71	0.55	0.0676	1.49	5
TN (%)	None	0.12	0.78	0.13	0.71	0.0206	1.86	8
	Mean norm	0.12	0.79	0.12	0.72	0.0087	1.90	8
	SNV	0.12	0.77	0.12	0.73	0.0183	1.91	7
	1st der	0.10	0.86	0.12	0.75	0.0075	1.98	8
	2nd der	0.10	0.85	0.11	0.77	-0.0002	2.10	6
	1st der & SNV	0.10	0.85	0.11	0.76	0.0030	2.05	7
	2nd der & SNV	0.11	0.83	0.11	0.79	0.0073	2.18	5
TSN (%)	None	0.05	0.82	0.08	0.45	0.0069	1.33	13
	Mean norm	0.05	0.80	0.08	0.45	0.0016	1.34	12
	SNV	0.05	0.82	0.08	0.48	0.0081	1.38	12
	1st der	0.04	0.86	0.07	0.55	0.0159	1.49	10
	2nd der	0.04	0.87	0.07	0.55	0.0104	1.47	8
	1st der & SNV	0.04	0.87	0.07	0.54	0.0127	1.47	10
	2nd der & SNV	0.05	0.85	0.07	0.53	0.0097	1.46	8
KI	None	3.38	0.20	3.13	0.18	0.2757	1.09	7
	Mean norm	3.40	0.19	3.10	0.18	0.3094	1.10	6
	SNV	3.43	0.18	3.11	0.18	0.2215	1.09	5
	1st der	3.52	0.13	3.18	0.13	0.1716	1.07	3
	2nd der	3.19	0.13	3.20	0.12	0.4276	1.06	3
	1st der & SNV	3.42	0.14	3.11	0.16	0.1458	1.09	2
	2nd der & SNV	3.63	0.11	3.15	0.14	0.2490	1.08	2
FAN (mg/L)	None	33.41	0.20	26.26	0.36	1.7792	1.25	6
	Mean norm	33.71	0.13	26.71	0.35	0.1817	1.23	4
	SNV	34.31	0.18	26.15	0.36	1.5392	1.25	4
	1st der	36.06	0.15	28.00	0.27	1.2489	1.17	2
	2nd der	35.38	0.18	28.01	0.27	-2.3490	1.17	2
	1st der & SNV	33.57	0.26	29.17	0.26	3.1270	1.12	3
	2nd der & SNV	36.25	0.14	28.78	0.23	-2.6576	1.14	1

Table 7 continued

DP (W.K.)	None	83.92	0.51	71.76	0.54	-7.3329	1.46	4
	Mean norm	83.76	0.52	71.57	0.54	-7.3677	1.47	3
	SNV	84.19	0.51	72.90	0.53	-7.1817	1.45	2
	1^{st} der	81.48	0.54	70.46	0.55	-6.6961	1.49	2
	2^{nd} der	71.64	0.65	69.68	0.57	-1.8927	1.50	4
	1^{st} der & SNV	78.11	0.58	72.23	0.53	-1.2279	1.45	3
	2^{nd} der & SNV	68.30	0.68	70.74	0.56	0.5031	1.48	4
Viscosity (cP)	None	0.02	0.29	0.02	0.29	-0.0003	1.17	4
	Mean norm	0.02	0.28	0.02	0.29	-0.0004	1.18	3
	SNV	0.02	0.28	0.02	0.30	-0.0003	1.18	2
	1^{st} der	0.02	0.27	0.02	0.27	0.0011	1.15	2
	2^{nd} der	0.02	0.28	0.02	0.30	0.0027	1.17	2
	1^{st} der & SNV	0.02	0.36	0.02	0.30	0.0022	1.16	3
	2^{nd} der & SNV	0.02	0.44	0.02	0.34	0.0023	1.21	4
AAL	None	1.32	0.60	1.60	0.26	-0.0774	1.12	10
	Mean norm	1.29	0.62	1.60	0.25	0.0242	1.13	10
	SNV	1.59	0.41	1.64	0.20	0.0905	1.10	4
	1^{st} der	1.49	0.48	1.63	0.21	0.0479	1.11	5
	2^{nd} der	1.49	0.48	1.63	0.22	-0.0383	1.10	4
	1^{st} der & SNV	1.53	0.46	1.64	0.20	0.0976	1.10	4
	2^{nd} der & SNV	1.46	0.51	1.64	0.21	0.0205	1.10	4
β-glucans (mg/L)	None	14.47	0.28	15.55	0.27	-3.4185	1.85	7
	Mean norm	15.56	0.16	14.22	0.22	-2.0624	2.02	3
	SNV	14.47	0.25	15.42	0.28	-2.4586	1.87	7
	1^{st} der	14.35	0.17	15.45	0.29	-3.9807	1.86	4
	2^{nd} der	16.90	0.18	15.81	0.24	-0.8805	1.82	3
	1^{st} der & SNV	14.72	0.13	14.12	0.24	-1.9113	2.04	3
	2^{nd} der & SNV	15.66	0.29	15.65	0.27	-1.1602	1.84	4

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1^{st} der=Savitzky-Golay first derivative, 9 points; 2^{nd} der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 8 Calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for dry land whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.47	0.75	0.50		0.72	0.0100	1.85	8
	Test set	none	0.55	0.66		0.70	0.45	-0.0204	1.33	7
	CV	2nd der	0.59	0.61	0.63		0.55	-0.0022	1.46	3
	Test set	2nd der	0.62	0.59		0.59	0.60	0.0462	1.58	3
Plumpness (%)	CV	none	3.01	0.34	3.21		0.26	-0.0035	1.26	6
	Test set	none	2.97	0.33		3.34	0.33	0.3840	1.21	5
	CV	2nd der	3.19	0.31	3.31		0.26	-0.0083	1.22	2
	Test set	2nd der	3.18	0.30		3.31	0.29	-0.0436	1.22	2
Extract (%)	CV	1st der	0.74	0.60	0.78		0.56	0.0030	1.37	3
	Test set	1st der	0.77	0.60		0.68	0.60	0.1131	1.55	3
	CV	2nd der	0.69	0.65	0.74		0.60	0.0039	1.43	3
	Test set	2nd der	0.71	0.67		0.67	0.62	0.0082	1.59	3
TN (%)	CV	1st der	0.10	0.83	0.11		0.79	0.0006	2.05	5
	Test set	1st der	0.11	0.83		0.11	0.77	0.0071	2.08	5
	CV	2nd der	0.10	0.84	0.11		0.81	0.0012	2.11	4
	Test set	2nd der	0.11	0.83		0.10	0.81	0.0131	2.26	3
TSN (%)	CV	1st der	0.06	0.75	0.07		0.64	-0.0003	1.51	7
	Test set	1st der	0.07	0.68		0.07	0.53	-0.0003	1.46	7
	CV	2nd der	0.06	0.72	0.07		0.65	-0.0007	1.55	4
	Test set	2nd der	0.06	0.72		0.07	0.52	0.0065	1.50	4
KI	CV	none	3.49	0.09	3.59		0.04	-0.0063	0.95	2
	Test set	none	3.61	0.09		3.28	0.07	0.1013	1.04	2
	CV	mean norm	3.47	0.09	3.57		0.05	0.0014	0.95	1
	Test set	mean norm	3.63	0.07		3.23	0.10	0.1057	1.05	1
FAN (mg/L)	CV	none	25.13	0.51	27.62		0.41	-0.0061	1.18	7
	Test set	none	28.13	0.44		26.72	0.36	9.0577	1.22	6
	CV	SNV	24.87	0.53	26.98		0.45	-0.1836	1.21	6
	Test set	SNV	26.44	0.52		21.93	0.52	8.9617	1.49	5
DP (W.K.)	CV	1st der	63.92	0.69	70.13		0.64	0.3593	1.51	6
	Test set	1st der	67.84	0.68		58.52	0.68	-1.2760	1.81	5
	CV	2nd der	71.06	0.62	75.09		0.71	-0.0419	1.41	3
	Test set	2nd der	72.77	0.63		69.70	0.73	-1.8026	1.52	3
Viscosity (cP)	CV	SNV	0.02	0.32	0.02		0.27	-0.0001	1.03	3
	Test set	SNV	0.02	0.33		0.02	0.30	-0.0001	1.19	3
	CV	2nd der	0.02	0.35	0.02		0.32	-0.0001	1.07	1
	Test set	2nd der	0.02	0.37		0.02	0.35	0.0019	1.22	1
AAL	CV	none	1.69	0.28	1.76		0.23	-0.0037	1.03	3
	Test set	none	1.68	0.35		1.74	0.13	0.0939	1.03	3
	CV	mean norm	1.34	0.55	1.50		0.45	-0.0081	1.20	7
	Test set	Mean norm	1.62	0.40		1.65	0.22	-0.0026	1.09	3
β-glucans (mg/L)	CV	SNV	16.11	0.12	16.54		0.08	0.0116	1.74	2
	Test set	SNV	16.03	0.08		16.40	0.20	-2.6770	1.75	2
	CV	1st der	14.66	0.22	15.21		0.16	0.1297	1.89	3
	Test set	1st der	15.19	0.07		16.35	0.24	-2.9668	1.76	2

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 9 Summary of calibration and test set validation results for models from the Büchi NIRLab N-200 data for irrigation whole grain samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r²	Bias	RPD	PLS
Moisture (%)	None	0.42	0.02	0.67	0.07	-0.0825	0.94	11
	Mean norm	0.42	0.03	0.68	0.05	-0.1037	0.93	11
	SNV	0.46	0.01	0.69	0.05	-0.0259	0.91	9
	1st der	0.58	0.07	0.63	0.08	-0.0491	1.00	4
	2nd der	0.38	0.06	0.72	0.04	-0.0944	0.88	7
	1st der & SNV	0.58	0.19	0.66	0.04	-0.0381	0.95	3
	2nd der & SNV	0.36	0.17	0.76	0.03	-0.0826	0.82	7
Plumpness (%)	None	3.66	0.54	3.26	0.45	-1.2614	1.17	3
	Mean norm	3.66	0.54	3.07	0.47	-1.2508	1.25	3
	SNV	3.51	0.52	3.27	0.43	-1.5400	1.17	3
	1st der	3.26	0.60	3.22	0.51	-1.2237	1.19	4
	2nd der	3.42	0.60	3.18	0.49	-0.9405	1.20	3
	1st der & SNV	3.50	0.58	3.10	0.52	-1.3061	1.23	3
	2nd der & SNV	3.68	0.53	3.19	0.48	-1.2616	1.20	2
Extract (%)	None	1.21	0.03	0.91	0.27	0.0404	1.08	2
	Mean norm	1.22	0.02	0.92	0.20	0.0186	1.06	1
	SNV	1.17	0.10	0.90	0.15	0.1293	1.08	3
	1st der	1.20	0.05	0.88	0.33	0.0607	1.11	1
	2nd der	1.20	0.05	0.87	0.34	0.0719	1.12	1
	1st der & SNV	1.19	0.07	0.89	0.22	0.1483	1.10	1
	2nd der & SNV	0.61	0.68	0.83	0.28	0.2181	1.17	6
TN (%)	None	0.15	0.38	0.16	0.16	0.0136	1.03	4
	Mean norm	0.15	0.35	0.15	0.15	0.0155	1.06	4
	SNV	0.15	0.36	0.15	0.17	0.0195	1.06	4
	1st der	0.11	0.68	0.15	0.23	0.0081	1.08	7
	2nd der	0.10	0.68	0.15	0.27	0.0252	1.06	5
	1st der & SNV	0.11	0.66	0.15	0.25	0.0162	1.09	6
	2nd der & SNV	0.11	0.67	0.15	0.25	0.0230	1.04	5
TSN (%)	None	0.11	0.11	0.10	0.0313	0.0114	0.97	1
	Mean norm	0.12	0.02	0.10	0.0004	0.0052	0.98	1
	SNV	0.12	0.03	0.10	0.0008	0.0006	0.96	1
	1st der	0.11	0.13	0.11	0.0050	-0.0004	0.94	1
	2nd der	0.11	0.14	0.11	0.0044	-0.0017	0.93	1
	1st der & SNV	0.11	0.10	0.10	0.0024	-0.0011	0.94	1
	2nd der & SNV	0.11	0.14	0.10	0.0094	-0.0040	0.94	1
KI	None	4.03	0.098	3.32	0.105	0.6046	1.06	1
	Mean norm	4.09	0.003	3.48	0.026	0.1007	1.01	1
	SNV	3.84	0.046	3.57	0.002	-0.1516	0.98	1
	1st der	4.08	0.077	3.81	0.002	0.0477	0.92	1
	2nd der	4.11	0.061	3.76	0.002	0.0249	0.93	1
	1st der & SNV	4.06	0.046	3.72	0.006	0.0458	0.94	1
	2nd der & SNV	3.93	0.094	3.83	0.0005	0.0848	0.92	1
FAN (mg/L)	None	35.05	0.096	30.87	0.13	-0.1981	1.07	2
	Mean norm	38.03	0.003	32.69	0.05	1.0511	1.01	1
	SNV	37.39	0.036	32.48	0.04	1.5630	1.02	2
	1st der	34.12	0.108	31.34	0.11	0.5117	1.06	1
	2nd der	37.60	0.025	31.55	0.11	0.5792	1.05	1
	1st der & SNV	37.54	0.028	31.42	0.11	-2.4869	1.05	1
	2nd der & SNV	37.09	0.051	31.17	0.11	-3.5760	1.06	1

Table 9 continued

DP (W.K.)	None	70.98	0.378	71.90	0.15	-5.6767	1.06	9
	Mean norm	84.30	0.016	75.06	0.03	-4.7005	1.01	2
	SNV	85.62	0.074	82.05	0.01	-1.6748	0.93	1
	1st der	85.09	0.045	82.28	0.04	-0.0231	0.92	1
	2nd der	84.55	0.042	81.47	0.04	-0.4215	0.93	1
	1st der & SNV	84.96	0.089	84.50	0.03	0.9040	0.90	1
	2nd der & SNV	86.90	0.067	82.54	0.04	1.2460	0.92	1
Viscosity (cP)	None	0.03	0.021	0.02	0.16	0.0040	1.07	3
	Mean norm	0.03	0.014	0.02	0.20	0.0032	1.06	2
	SNV	0.03	0.023	0.02	0.17	0.0023	1.07	1
	1st der	0.02	0.034	0.02	0.20	-0.0004	1.08	1
	2nd der	0.02	0.035	0.02	0.21	-0.0003	1.09	1
	1st der & SNV	0.03	0.044	0.02	0.25	0.0015	1.12	1
	2nd der & SNV	0.02	0.112	0.02	0.19	-0.0025	1.10	1
AAL	None	1.54	0.50	1.78	0.20	0.2557	1.07	9
	Mean norm	2.13	0.04	2.10	0.12	0.1156	0.91	1
	SNV	2.09	0.22	1.77	0.16	-0.0706	1.08	4
	1st der	2.31	0.02	2.02	0.08	-0.0495	0.94	4
	2nd der	2.30	0.03	2.06	0.08	-0.0488	0.92	1
	1st der & SNV	2.08	0.07	2.09	0.04	0.0739	0.91	1
	2nd der & SNV	1.08	0.62	1.66	0.14	0.1901	1.15	5
β-glucans (mg/L)	None	44.47	0.15	46.48	0.13	-7.5331	1.57	5
	Mean norm	43.84	0.18	44.83	0.19	-8.5266	1.63	5
	SNV	46.40	0.07	41.16	0.18	-2.5410	1.78	2
	1st der	45.74	0.09	44.56	0.11	-1.1959	1.64	2
	2nd der	46.78	0.05	47.21	0.24	-5.5154	1.55	1
	1st der & SNV	44.96	0.12	45.19	0.20	-4.3837	1.62	2
	2nd der & SNV	45.68	0.11	44.14	0.23	-2.2988	1.66	1

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 10 Calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for irrigation whole grain samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.58	0.11	0.63		0.11	-0.0119	1.00	5
	Test set	none	0.67	0.00		0.62	0.004	0.0568	1.00	7
	CV	1st der	0.55	0.13	0.62		0.13	-0.0045	1.01	4
	Test set	1st der	0.42	0.07		0.66	0.07	-0.0825	0.94	4
Plumpness	CV	none	3.57	0.49	3.67		0.46	0.0130	1.04	2
(%)	Test set	none	3.69	0.53		3.33	0.44	-1.4478	1.15	2
	CV	1st der	2.96	0.65	3.14		0.60	0.0347	1.22	4
	Test set	1st der	3.04	0.68		3.00	0.56	-0.8338	1.28	4
Extract (%)	CV	1st der	1.10	0.08	1.13		0.04	-0.0043	0.86	1
	Test set	1st der	1.20	0.05		0.88	0.32	0.0676	1.11	1
	CV	2nd der	1.10	0.09	1.13		0.04	-0.0043	0.86	1
	Test set	2nd der	1.20	0.05		0.87	0.34	0.0755	1.12	1
TN (%)	CV	1st der	0.13	0.30	0.15		0.30	-0.0010	1.04	4
	Test set	1st der	0.16	0.09		0.16	0.09	0.0450	0.98	1
	CV	2nd der	0.13	0.38	0.14		0.38	0.00001	1.16	2
	Test set	2nd der	0.13	0.46		0.17	0.10	0.0147	0.94	2
TSN (%)	CV	2nd der	0.10	0.27	0.11		0.11	0.0016	0.91	3
	Test set	2nd der	0.11	0.09		0.11	0.002	-0.0018	0.93	1
	CV	1st der	0.10	0.23	0.11		0.10	-0.0002	0.91	3
	Test set	1st der	0.11	0.16		0.10	0.04	0.0159	1.01	1
KI	CV	none	3.82	0.093	3.88		0.07	-0.0080	0.90	1
	Test set	none	4.03	0.098		3.32	0.11	0.6046	1.06	1
	CV	mean norm	3.30	0.281	3.67		0.14	0.0031	0.95	6
	Test set	mean norm	4.08	0.004		3.47	0.04	0.1103	1.01	1
FAN (mg/L)	CV	none	33.60	0.106	34.64		0.06	0.0633	0.96	2
	Test set	none	35.10	0.094		30.98	0.12	-0.3196	1.07	2
	CV	2nd der	32.82	0.189	33.84		0.14	-0.0214	0.98	1
	Test set	2nd der	33.55	0.224		31.49	0.22	-6.2397	1.05	1
DP (W.K.)	CV	none	85.82	0.001	88.50		0.19	-0.7112	0.86	1
	Test set	none	70.98	0.378		71.90	0.15	-5.6767	1.06	1
	CV	mean norm	81.37	0.012	83.44		0.01	-0.1265	0.91	1
	Test set	mean norm	84.30	0.016		75.06	0.03	-4.7005	1.01	1
Viscosity (cP)	CV	mean norm	0.03	0.005	0.03		0.04	0.0000	0.79	1
	Test set	mean norm	0.02	0.07		0.02	0.29	0.0030	1.08	1
	CV	2nd der	0.02	0.070	0.02		0.03	0.0000	0.92	1
	Test set	2nd der	0.02	0.035		0.02	0.22	-0.0003	1.09	1
AAL	CV	none	2.03	0.05	2.10		0.01	-0.0180	0.91	1
	Test set	none	2.09	0.08		1.96	0.01	-0.0773	0.98	1
	CV	SNV	1.95	0.23	2.14		0.10	0.0152	0.89	5
	Test set	SNV	2.25	0.09		1.85	0.07	-0.0750	1.03	4
β-glucans	CV	mean norm	48.12	0.02	48.56		0.01	0.1812	1.51	1
(mg/L)	Test set	mean norm	46.74	0.07		47.87	0.03	0.2319	1.53	1
	CV	2nd der	46.74	0.07	47.87		0.03	0.2320	1.53	1
	Test set	2nd der	47.75	0.01		46.57	0.30	-4.3962	1.57	1

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 11 Summary of calibration and validation results for test set validation models from the Büchi NIRLab N-200 data for dry land flour samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture (%)	None	0.46	0.21	0.46	0.76	0.0172	2.03	6
	Mean norm	0.47	0.75	0.50	0.72	-0.0072	1.88	3
	SNV	0.47	0.73	0.54	0.67	-0.0938	1.72	2
	1st der	0.41	0.80	0.52	0.69	-0.0302	1.79	4
	2nd der	0.43	0.69	0.53	0.69	-0.0740	1.77	3
	1st der & SNV	0.44	0.78	0.54	0.69	-0.0874	1.73	1
	2nd der & SNV	0.44	0.69	0.60	0.60	-0.0287	1.54	2
Plumpness	None	2.23	0.01	4.14	0.13	0.6521	0.97	12
(%)	Mean norm	2.25	0.01	4.16	0.14	0.8644	0.97	12
	SNV	1.83	0.02	4.42	0.10	0.4706	0.91	13
	1st der	2.08	0.67	3.06	0.34	-0.3157	1.32	8
	2nd der	2.46	0.57	3.36	0.23	-0.7848	1.20	7
	1st der & SNV	2.31	0.62	3.46	0.17	-0.5931	1.17	8
	2nd der & SNV	2.06	0.04	3.75	0.14	-0.8264	1.08	8
Extract (%)	None	0.77	0.60	0.82	0.44	0.0646	1.30	6
	Mean norm	0.80	0.57	0.80	0.45	0.0131	1.32	5
	SNV	0.72	0.62	0.82	0.43	0.1424	1.30	2
	1st der	0.58	0.76	0.81	0.48	0.1777	1.32	3
	2nd der	0.76	0.61	0.84	0.43	0.0465	1.27	2
	1st der & SNV	0.76	0.61	0.82	0.44	0.0856	1.30	2
	2nd der & SNV	0.82	0.55	0.84	0.42	-0.0157	1.27	2
TN (%)	None	0.12	0.80	0.10	0.83	-0.0247	2.40	6
	Mean norm	0.12	0.79	0.09	0.84	-0.0249	2.49	5
	SNV	0.10	0.86	0.09	0.84	-0.0130	2.51	7
	1st der	0.10	0.85	0.10	0.83	-0.0213	2.39	4
	2nd der	0.10	0.84	0.10	0.81	-0.0185	2.30	4
	1st der & SNV	0.10	0.85	0.10	0.82	-0.0200	2.34	4
	2nd der & SNV	0.11	0.83	0.10	0.81	-0.0239	2.30	2
TSN (%)	None	0.07	0.68	0.07	0.54	0.0055	1.47	5
	Mean norm	0.06	0.72	0.07	0.58	0.0060	1.54	5
	SNV	0.06	0.72	0.07	0.56	0.0073	1.50	4
	1st der	0.06	0.71	0.07	0.58	0.0073	1.55	4
	2nd der	0.06	0.73	0.07	0.58	0.0041	1.54	4
	1st der & SNV	0.07	0.64	0.07	0.57	-0.0037	1.53	2
	2nd der & SNV	0.07	0.68	0.07	0.59	0.0003	1.56	2
KI	None	3.58	0.14	3.22	0.13	0.2916	1.06	4
	Mean norm	3.50	0.13	3.14	0.15	0.3529	1.08	3
	SNV	3.20	0.29	3.15	0.20	0.3165	1.08	6
	1st der	3.64	0.12	3.30	0.08	0.1581	1.03	2
	2nd der	3.53	0.17	3.23	0.11	0.1357	1.05	2
	1st der & SNV	3.44	0.19	3.24	0.12	0.2303	1.05	2
	2nd der & SNV	3.61	0.13	3.22	0.11	0.1257	1.06	1
FAN (mg/L)	None	28.89	0.45	21.01	0.59	4.9237	1.56	6
	Mean norm	29.61	0.43	21.70	0.56	3.8346	1.51	5
	SNV	29.84	0.42	21.27	0.58	2.1117	1.54	4
	1st der	29.82	0.42	21.03	0.60	5.1192	1.56	3
	2nd der	28.33	0.48	22.26	0.54	4.7426	1.47	4
	1st der & SNV	29.36	0.44	21.31	0.58	5.2725	1.54	3
	2nd der & SNV	30.67	0.38	22.63	0.53	4.1325	1.45	1

Table 11 continued

DP (W.K.)	None	84.66	0.49	82.71	0.40	-1.3232	1.28	3
	Mean norm	78.98	0.55	82.96	0.42	-6.4368	1.27	5
	SNV	71.99	0.63	77.89	0.48	-12.0018	1.36	6
	1st der	73.21	0.61	79.07	0.47	-0.0347	1.34	4
	2nd der	83.09	0.52	89.50	0.36	6.8429	1.18	3
	1st der & SNV	74.04	0.61	80.38	0.46	4.2296	1.32	4
	2nd der & SNV	86.35	0.49	83.57	0.40	4.0616	1.27	2
Viscosity (cP)	None	0.02	0.45	0.02	0.29	0.0031	1.13	8
	Mean norm	0.02	0.26	0.02	0.26	0.0022	1.16	2
	SNV	0.02	0.29	0.02	0.31	0.0022	1.19	3
	1st der	0.02	0.49	0.02	0.41	0.0038	1.27	5
	2nd der	0.02	0.55	0.02	0.43	0.0029	1.22	5
	1st der & SNV	0.02	0.25	0.02	0.32	0.0012	1.21	1
	2nd der & SNV	86.35	0.49	83.57	0.40	4.0616	0.00026	2
AAL	None	1.57	0.43	1.75	0.12	0.0989	1.03	4
	Mean norm	1.60	0.41	1.67	0.18	0.1621	1.08	4
	SNV	1.68	0.33	1.59	0.22	0.2175	1.13	2
	1st der	1.45	0.51	1.66	0.19	-0.0437	1.08	4
	2nd der	1.51	0.47	1.58	0.25	0.0773	1.14	4
	1st der & SNV	1.45	0.51	1.66	0.21	-0.0857	1.09	4
	2nd der & SNV	1.66	0.36	1.65	0.16	0.2447	1.09	2
β-glucans	None	17.04	0.17	16.10	0.22	-0.9658	1.79	4
(mg/L)	Mean norm	14.99	0.23	15.69	0.25	0.0913	1.83	5
	SNV	14.42	0.38	16.08	0.24	-0.4528	1.79	7
	1st der	16.58	0.21	15.96	0.23	-0.0370	1.80	3
	2nd der	17.38	0.13	16.04	0.23	0.6047	1.79	2
	1st der & SNV	16.96	0.17	15.98	0.23	0.2764	1.80	2
	2nd der & SNV	15.93	0.28	15.60	0.26	1.3916	1.84	2

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 12 Calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for dry land flour samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.85	0.73	0.51		0.70	-0.0012	1.81	3
	Test set	none	0.49	0.73		0.52	0.69	0.0577	1.79	3
	CV	mean norm	0.86	0.73	0.49		0.72	-0.0010	1.88	2
	Test set	mean norm	0.47	0.74		0.51	0.71	0.0197	1.82	2
Plumpness (%)	CV	1st der	2.53	0.51	2.84		0.39	-0.0274	1.42	7
	Test set	1st der	2.56	0.50		2.75	0.45	0.2029	1.47	7
	CV	2nd der	2.45	0.01	3.75		0.12	0.0185	1.08	8
	Test set	2nd der	2.46	0.57		3.36	0.23	-0.7848	1.20	7
Extract (%)	CV	mean norm	0.81	0.52	0.84		0.48	-0.0048	1.27	2
	Test set	mean norm	0.81	0.56		0.82	0.43	-0.0031	1.30	2
	CV	1st der	0.68	0.64	0.70		0.62	-0.0018	1.52	2
	Test set	1st der	0.62	0.73		0.87	0.42	0.1434	1.22	2
TN (%)	CV	mean norm	0.11	0.81	0.12		0.79	0.0005	2.01	5
	Test set	mean norm	0.12	0.78		0.09	0.84	-0.0210	2.53	5
	CV	SNV	0.10	0.84	0.11		0.82	0.0003	2.17	5
	Test set	SNV	0.11	0.83		0.09	0.84	-0.0237	2.46	4
TSN (%)	CV	1st der	0.07	0.67	0.07		0.64	-0.0001	1.53	3
	Test set	1st der	0.07	0.63		0.07	0.58	0.0097	1.55	2
	CV	2nd der	0.06	0.68	0.07		0.65	0.0001	1.58	3
	Test set	2nd der	0.06	0.72		0.07	0.61	0.0048	1.59	3
KI	CV	mean norm	3.32	0.17	3.59		0.06	-0.0110	0.95	4
	Test set	mean norm	3.60	0.09		3.25	0.09	0.2836	1.05	3
	CV	SNV	3.19	0.24	3.39		0.15	0.0206	1.00	4
	Test set	SNV	3.37	0.21		3.07	0.20	0.3861	1.11	3
FAN (mg/L)	CV	none	28.30	0.41	29.73		0.35	-0.0545	1.10	2
	Test set	none	30.56	0.38		23.04	0.51	1.7617	1.42	2
	CV	1st der	26.92	0.47	28.17		0.53	-0.1367	1.16	3
	Test set	1st der	29.13	0.45		21.23	0.58	5.8125	1.54	3
DP (W.K.)	CV	SNV	73.89	0.58	78.21		0.53	0.0127	1.35	5
	Test set	SNV	72.98	0.62		77.35	0.49	-10.1074	1.37	5
	CV	1st der	73.63	0.58	78.31		0.53	0.1594	1.35	4
	Test set	1st der	72.08	0.63		79.22	0.47	1.5111	1.34	5
Viscosity (cP)	CV	1st der	0.26	0.26	0.02		0.49	-0.00003	1.01	1
	Test set	1st der	0.02	0.28		0.02	0.24	0.0001	1.09	1
	CV	2nd der	0.41	0.41	0.02		0.59	0.0001	1.09	3
	Test set	2nd der	0.38	0.38		0.02	0.65	0.0039	1.30	2
AAL	CV	SNV	1.54	0.39	1.63		0.32	0.0019	1.10	4
	Test set	SNV	1.57	0.42		1.60	0.21	0.0501	1.12	2
	CV	2nd der	1.65	0.31	1.73		0.25	0.0032	1.04	2
	Test set	2nd der	1.60	0.41		1.55	0.26	0.0652	1.16	2
β-glucans (mg/L)	CV	mean norm	14.38	0.32	15.43		0.23	-0.0024	1.86	6
	Test set	mean norm	14.32	0.30		15.41	0.28	-0.2478	1.87	4
	CV	SNV	15.00	0.32	15.39		0.25	0.0228	1.87	5
	Test set	SNV	15.36	0.30		14.64	0.35	1.6123	1.96	5

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 13 Summary of calibration and test set validation results for models from the Büchi NIRLab N-200 data for irrigation flour samples

Property	Pretreatment	Calibration		Validation				
		SEC	R^2	SEP	r^2	Bias	RPD	PLS
Moisture (%)	None	0.29	0.80	0.35	0.69	0.0488	1.81	6
	Mean norm	0.30	0.79	0.35	0.69	0.0510	1.78	5
	SNV	0.41	0.58	0.39	0.61	0.0848	1.59	2
	1st der	0.28	0.77	0.38	0.64	0.0120	1.65	4
	2nd der	0.29	0.75	0.69	0.62	0.0207	0.91	4
	1st der & SNV	0.33	0.68	0.40	0.62	0.0155	1.59	1
	2nd der & SNV	0.30	0.74	0.40	0.61	0.0274	1.59	3
Plumpness (%)	None	3.93	0.43	2.75	0.49	-1.6425	1.39	7
	Mean norm	3.99	0.45	2.80	0.45	-1.8182	1.37	6
	SNV	3.98	0.46	2.74	0.50	-1.8293	1.40	5
	1st der	3.46	0.59	2.91	0.50	-1.3335	1.32	5
	2nd der	3.65	0.54	3.02	0.46	-1.4678	1.27	3
	1st der & SNV	4.23	0.39	2.97	0.43	-1.9087	1.29	1
	2nd der & SNV	3.89	0.48	2.90	0.46	-2.0018	1.32	2
Extract (%)	None	0.42	0.89	0.72	0.55	0.0911	1.36	10
	Mean norm	0.22	0.97	0.73	0.57	0.0438	1.33	13
	SNV	0.81	0.40	0.73	0.46	0.1614	1.34	3
	1st der	0.59	0.72	0.75	0.45	0.1086	1.30	6
	2nd der	0.84	0.47	0.86	0.30	0.2069	1.13	2
	1st der & SNV	0.78	0.60	0.83	0.33	0.2575	1.17	3
	2nd der & SNV	0.91	0.45	0.86	0.29	0.1565	1.13	1
TN (%)	None	0.06	0.88	0.10	0.62	-0.0069	1.61	11
	Mean norm	0.10	0.69	0.10	0.62	0.0001	1.61	7
	SNV	0.09	0.73	0.10	0.62	-0.0122	1.60	7
	1st der	0.09	0.78	0.10	0.65	-0.0093	1.68	5
	2nd der	0.09	0.75	0.10	0.60	-0.0096	1.57	3
	1st der & SNV	0.08	0.80	0.10	0.63	-0.0071	1.62	5
	2nd der & SNV	0.10	0.70	0.10	0.60	-0.0098	1.58	2
TSN (%)	None	0.08	0.38	0.06	0.59	-0.0014	1.56	6
	Mean norm	0.08	0.35	0.06	0.62	-0.0025	1.61	6
	SNV	0.08	0.34	0.06	0.61	-0.0023	1.59	5
	1st der	0.07	0.42	0.07	0.52	0.0012	1.41	4
	2nd der	0.07	0.48	0.08	0.46	0.0081	1.25	4
	1st der & SNV	0.09	0.46	0.07	0.48	0.0111	1.33	4
	2nd der & SNV	0.08	0.37	0.08	0.39	0.0032	1.25	2
KI	None	3.68	0.15	3.34	0.11	-0.3358	1.05	4
	Mean norm	3.07	0.42	2.99	0.30	-0.5067	1.17	7
	SNV	3.06	0.43	2.88	0.34	-0.4748	1.22	7
	1st der	3.23	0.42	3.01	0.31	-0.4265	1.16	5
	2nd der	3.18	0.44	3.04	0.30	-0.1733	1.16	4
	1st der & SNV	2.98	0.46	2.85	0.37	-0.4232	1.23	4
	2nd der & SNV	3.26	0.41	2.74	0.39	-0.1803	1.28	3
FAN (mg/L)	None	22.20	0.60	26.73	0.36	-3.9499	1.24	9
	Mean norm	5.40	0.54	22.86	0.54	-1.1544	1.45	13
	SNV	19.76	0.71	25.56	0.41	-5.8003	1.30	8
	1st der	30.43	0.36	26.92	0.34	-0.8869	1.23	3
	2nd der	32.99	0.25	27.71	0.30	-1.7630	1.19	2
	1st der & SNV	28.28	0.39	27.04	0.34	-0.4635	1.22	2
	2nd der & SNV	27.63	0.41	27.35	0.32	-0.4685	1.21	2

Table 13 continued

DP (W.K.)	None	41.72	0.74	51.22	0.55	13.2325	1.48	10
	Mean norm	47.71	0.67	49.28	0.58	5.5782	1.54	7
	SNV	54.65	0.57	54.70	0.48	3.4284	1.39	6
	1st der	67.14	0.26	60.12	0.38	-0.5052	1.26	2
	2nd der	67.08	0.31	57.58	0.43	1.0552	1.32	2
	1st der & SNV	29.04	0.36	56.30	0.45	0.2538	1.35	2
	2nd der & SNV	70.37	0.35	57.96	0.43	3.1676	1.31	2
Viscosity (cP)	None	0.01	0.59	0.02	0.47	-0.0021	1.37	10
	Mean norm	0.02	0.43	0.02	0.39	-0.0009	1.27	7
	SNV	0.02	0.40	0.02	0.43	-0.0011	1.31	6
	1st der	0.02	0.53	0.02	0.28	0.0006	1.18	5
	2nd der	0.02	0.27	0.02	0.37	0.0038	1.26	3
	1st der & SNV	0.02	0.53	0.02	0.41	-0.0003	1.28	5
	2nd der & SNV	0.03	0.19	0.02	0.27	0.0033	1.17	1
AAL	None	1.94	0.14	1.93	0.03	-0.0319	0.99	1
	Mean norm	1.92	0.32	1.70	0.23	-0.4187	1.12	7
	SNV	1.95	0.30	1.73	0.21	-0.3268	1.10	6
	1st der	2.31	0.02	1.90	0.01	-0.0740	1.00	1
	2nd der	1.60	0.12	1.87	0.06	0.2863	1.02	1
	1st der & SNV	2.15	0.15	2.02	0.02	-0.2471	0.95	1
	2nd der & SNV	2.12	0.18	2.07	0.01	-0.2409	0.92	1
β-glucans (mg/L)	None	44.48	0.16	40.37	0.42	-9.2572	1.81	5
	Mean norm	43.26	0.20	39.83	0.42	-8.1835	1.84	5
	SNV	44.47	0.16	40.78	0.41	-8.6053	1.79	4
	1st der	35.07	0.47	38.39	0.42	-3.0195	1.90	4
	2nd der	41.58	0.26	41.03	0.33	-3.9177	1.78	3
	1st der & SNV	43.54	0.18	40.92	0.38	-6.0963	1.79	2
	2nd der & SNV	36.73	0.39	41.64	0.39	-2.9118	1.76	3

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration); r^2=coefficient of determination (validation); SEC= standard error of calibration; SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectral pretreatment

Table 14 Calibration and validation results for uncertainty tested models from the Büchi NIRLab N-200 data for irrigation flour samples

Property	Validation	Pre-treatment	Calibration		Validation					
			SEC	R^2	SECV	SEP	r^2	Bias	RPD	PLS
Moisture (%)	CV	none	0.31	0.77	0.33		0.73	0.0018	1.88	6
	Test set	none	0.31	0.77		0.34	0.70	0.0625	1.82	4
	CV	mean norm	0.32	0.75	0.34		0.72	-0.0013	1.84	5
	Test set	mean norm	0.30	0.79		0.36	0.68	0.0430	1.76	5
Plumpness (%)	CV	none	4.11	0.35	4.29		0.30	0.0270	0.90	3
	Test set	none	4.47	0.35		3.05	0.40	-2.2555	1.26	3
	CV	1st der	3.89	0.39	4.02		0.35	0.0224	0.95	2
	Test set	1st der	4.39	0.34		3.10	0.39	-1.7600	1.23	1
Extract (%)	CV	none	0.53	0.79	0.63		0.71	0.0158	1.56	8
	Test set	none	0.55	0.81		0.66	0.58	0.1184	1.49	7
	CV	mean norm	0.70	0.63	0.76		0.57	0.0080	1.29	4
	Test set	mean norm	0.70	0.68		0.76	0.48	0.1372	1.28	4
TN (%)	CV	mean norm	0.11	0.61	0.12		0.57	-0.0009	1.35	5
	Test set	mean norm	0.12	0.62		0.11	0.53	-0.0063	1.45	5
	CV	1st der	0.10	0.67	0.11		0.64	-0.0007	1.50	3
	Test set	1st der	0.10	0.69		0.10	0.61	-0.0056	1.60	3
TSN (%)	CV	mean norm	0.43	0.43	0.08		0.38	0.0001	1.30	6
	Test set	mean norm	0.34	0.34		0.06	0.59	-0.0048	1.54	4
	CV	SNV	0.45	0.45	0.08		0.35	0.0000	1.27	5
	Test set	SNV	0.07	0.07		0.08	0.32	-0.0118	1.18	3
KI	CV	SNV	2.94	0.42	3.10		0.36	0.0533	1.13	4
	Test set	SNV	3.92	0.07		3.43	0.04	-0.3199	1.02	1
	CV	1st der	3.51	0.24	3.69		0.16	0.0175	0.95	2
	Test set	1st der	3.83	0.20		2.81	0.34	-0.4112	1.25	2
FAN (mg/L)	CV	mean norm	20.41	0.63	22.75		0.54	0.0864	1.45	6
	Test set	mean norm	19.97	0.65		23.82	0.48	-3.2745	1.39	4
	CV	SNV	21.84	0.62	23.73		0.55	0.0054	1.39	6
	Test set	SNV	21.26	0.66		23.78	0.49	-4.3789	1.39	6
DP (W.K.)	CV	none	52.36	0.57	63.36		0.39	0.8074	1.20	8
	Test set	none	77.01	0.11		71.17	0.11	-2.1780	1.05	3
	CV	mean norm	47.34	0.65	55.51		0.52	-0.4862	1.37	7
	Test set	mean norm	61.05	0.45		55.92	0.44	2.7709	1.34	3
Viscosity (cP)	CV	none	0.02	0.44	0.02		0.28	0.0001	1.13	6
	Test set	none	0.02	0.35		0.02	0.44	-0.0016	1.32	5
	CV	SNV	0.02	0.43	0.02		0.25	0.0001	1.17	6
	Test set	SNV	0.02	0.35		0.02	0.42	-0.0022	1.29	6
AAL	CV	mean norm	1.85	0.29	2.16		0.10	-0.0190	0.89	7
	Test set	mean norm	2.05	0.23		1.74	0.18	-0.3574	1.10	4
	CV	SNV	1.88	0.27	2.18		0.08	0.0133	0.88	6
	Test set	SNV	2.28	0.05		1.85	0.06	-0.0301	1.03	1
β-glucans (mg/L)	CV	mean norm	36.06	0.45	43.95		0.22	-0.2407	1.66	7
	Test set	mean norm	46.00	0.10		41.20	0.39	-4.9593	1.77	3
	CV	1st der	31.89	0.57	36.51		0.44	-0.9815	2.00	6
	Test set	1st der	34.81	0.48		33.83	0.54	-3.8780	2.16	6

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; CV=cross-validation; R^2=coefficient of determination (calibration or cross validation); SEC=standard error of calibration; SECV=standard error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; PLS=number of partial least squares factors; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative, 9 points; 2nd der=Savitzky-Golay second derivative, 17 points; Mean norm=mean normalization; none=no spectra pre-treatment

Table 15 Summary of the NIR prediction results for whole grain dry land barley as obtained with full cross-validation for combined 2008 and 2009 data, spectra recorded with the Büchi NIRLab N-200

Property	Pretreatment	Full cross-validation			
		PLS Factors	SECV	R^2	Bias
Plumpness (%)	none	12	3.93	0.50	0.0066
	mean norm	12	3.94	0.50	0.0056
	SNV	13	3.87	0.52	0.0170
	1st der	10	4.01	0.48	-0.0008
	2nd der	9	4.28	0.42	0.0124
	1st der + SNV	10	4.01	0.48	-0.0039
	2nd der + SNV	3	4.49	0.34	0.0089
Moisture (%)	none	15	0.69	0.41	0.0014
	mean norm	12	0.69	0.41	0.0037
	SNV	11	0.70	0.39	-0.0018
	1st der	9	0.70	0.40	0.0017
	2nd der	8	0.70	0.40	-0.0073
	1st der + SNV	5	0.71	0.37	0.0008
	2nd der + SNV	8	0.71	0.38	-0.00002
Extract (%)	none	11	1.25	0.39	0.0002
	mean norm	12	1.24	0.40	-0.0004
	SNV	11	1.25	0.39	0.0020
	1st der	8	1.22	0.41	-0.0011
	2nd der	7	1.25	0.39	0.00002
	1st der + SNV	8	1.22	0.42	-0.0002
	2nd der + SNV	7	1.26	0.38	-0.0002
TN (%)	none	12	0.21	0.58	0.0007
	mean norm	12	0.21	0.60	0.0005
	SNV	14	0.20	0.63	0.0003
	1st der	9	0.20	0.61	0.0003
	2nd der	7	0.21	0.58	0.0002
	1st der + SNV	8	0.20	0.61	0.0004
	2nd der + SNV	7	0.21	0.58	0.0003
TSN (%)	none	13	0.11	0.52	0.0006
	mean norm	12	0.11	0.52	0.0005
	SNV	9	0.12	0.49	0.0003
	1st der	9	0.11	0.54	0.0004
	2nd der	7	0.11	0.51	0.0002
	1st der + SNV	7	0.11	0.54	0.0004
	2nd der + SNV	5	0.12	0.49	0.0002
KI	none	8	5.92	0.35	0.0116
	mean norm	6	5.92	0.35	0.0217
	SNV	7	5.95	0.34	0.0128
	1st der	6	5.88	0.36	0.0376
	2nd der	7	5.85	0.37	0.0162
	1st der + SNV	7	5.84	0.37	0.0169
	2nd der + SNV	6	5.91	0.36	0.0094
FAN (mg/L)	none	13	34.84	0.36	0.1657
	mean norm	13	34.48	0.38	0.1158
	SNV	14	34.72	0.37	0.1259
	1st der	8	34.31	0.38	0.1584
	2nd der	7	34.50	0.37	0.0767
	1st der + SNV	8	34.17	0.38	0.0746
	2nd der + SNV	6	35.13	0.35	0.0893

Table 15 continued

DP (W.K.)	none	11	91.37	0.35	-0.7329
	mean norm	11	88.97	0.38	-0.5257
	SNV	9	93.14	0.32	0.3529
	1st der	8	91.42	0.35	0.0894
	2nd der	7	94.43	0.31	0.2371
	1st der + SNV	8	90.28	0.36	0.0737
	2nd der + SNV	7	93.84	0.32	0.2041
Viscosity (cP)	none	12	0.03	0.39	-0.000005
	mean norm	9	0.04	0.35	-0.000009
	SNV	8	0.04	0.34	-0.000003
	1st der	7	0.04	0.38	-0.000004
	2nd der	6	0.04	0.38	-0.00002
	1st der + SNV	7	0.04	0.36	0.000007
	2nd der + SNV	4	0.04	0.32	-0.00003
AAL	none	13	2.01	0.20	0.0011
	mean norm	3	2.12	0.08	-0.0008
	SNV	4	2.10	0.10	-0.0013
	1st der	2	2.07	0.13	-0.000002
	2nd der	3	2.10	0.10	-0.0009
	1st der + SNV	2	2.10	0.10	-0.0008
	2nd der + SNV	2	2.11	0.10	-0.0006
β-glucan (mg/L)	none	8	54.83	0.21	-0.3476
	mean norm	12	53.32	0.26	-0.2045
	SNV	11	53.58	0.26	-0.0897
	1st der	4	55.57	0.19	-0.0949
	2nd der	4	54.82	0.21	-0.1380
	1st der + SNV	3	55.49	0.19	-0.1066
	2nd der + SNV	3	54.68	0.22	-0.1144

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate; 1^{st} der=Savitzky-Golay first derivative; 2^{nd} der=Savitzky-Golay second derivative

Table 16 Summary of the NIR prediction results for ground dry land barley as obtained with full cross-validation for combined 2008 and 2009 data, spectra recorded with the Büchi NIRLab N-200

Property	Pretreatment	Full cross-validation			
		PLS Factors	SECV	R^2	Bias
Plumpness (%)	none	4	4.86	0.23	0.0042
	mean norm	11	4.49	0.35	-0.1107
	SNV	2	4.88	0.23	0.0059
	1st der	3	4.90	0.22	0.0034
	2nd der	6	4.70	0.29	0.0023
	1st der + SNV	6	4.74	0.28	0.0059
	2nd der + SNV	6	4.71	0.29	0.0050
Moisture (%)	none	4	0.57	0.59	0.0005
	mean norm	3	0.57	0.58	0.0002
	SNV	2	0.59	0.56	-0.0001
	1st der	4	0.57	0.59	0.0004
	2nd der	4	0.56	0.60	0.0007
	1st der + SNV	3	0.57	0.58	0.00002
	2nd der + SNV	3	0.57	0.59	0.0005
Extract (%)	none	11	1.20	0.43	0.0016
	mean norm	8	1.22	0.41	-0.0006
	SNV	9	1.22	0.42	0.0007
	1st der	2	1.29	0.35	-0.0004
	2nd der	2	1.28	0.36	-0.0009
	1st der + SNV	5	1.26	0.38	-0.0048
	2nd der + SNV	4	1.30	0.34	-0.0025
TN (%)	none	7	0.20	0.62	0.0003
	mean norm	6	0.20	0.62	0.0003
	SNV	5	0.20	0.61	0.0004
	1st der	4	0.20	0.62	0.0003
	2nd der	3	0.20	0.61	0.0003
	1st der + SNV	2	0.20	0.61	0.0004
	2nd der + SNV	1	0.20	0.60	0.0005
TSN (%)	none	4	0.12	0.44	-0.0002
	mean norm	7	0.12	0.46	0.0004
	SNV	9	0.12	0.50	-0.0001
	1st der	4	0.12	0.43	0.0003
	2nd der	3	0.13	0.41	0.0002
	1st der + SNV	4	0.12	0.42	0.0002
	2nd der + SNV	2	0.13	0.39	0.0002
KI	none	8	6.31	0.26	0.0093
	mean norm	9	6.24	0.28	0.0088
	SNV	8	6.30	0.27	0.0064
	1st der	4	6.38	0.25	-0.0070
	2nd der	4	6.41	0.24	0.0120
	1st der + SNV	6	6.30	0.27	0.0238
	2nd der + SNV	2	6.47	0.22	0.0041
FAN (mg/L)	none	12	39.16	0.25	0.1600
	mean norm	9	39.86	0.22	0.0712
	SNV	11	39.00	0.26	-0.0959
	1st der	4	40.77	0.17	-0.0286
	2nd der	2	41.43	0.14	0.0293
	1st der + SNV	5	39.27	0.19	0.3859
	2nd der + SNV	2	40.06	0.15	0.0428

Table 16 continued

DP (W.K.)	none	4	99.64	0.22	0.1949
	mean norm	4	99.84	0.22	0.1673
	SNV	8	95.86	0.28	-0.0037
	1st der	4	96.81	0.26	0.1522
	2nd der	4	97.62	0.25	0.1915
	1st der + SNV	4	96.66	0.27	0.0967
	2nd der + SNV	4	97.24	0.26	0.1071
Viscosity (cP)	none	13	0.04	0.31	0.00005
	mean norm	12	0.04	0.31	-0.00003
	SNV	11	0.04	0.27	0.0001
	1st der	9	0.04	0.29	0.00003
	2nd der	8	0.04	0.24	0.0001
	1st der + SNV	8	0.04	0.28	0.0001
	2nd der + SNV	8	0.04	0.25	0.0001
AAL	none	8	2.03	0.16	0.0028
	mean norm	7	2.06	0.15	0.0029
	SNV	6	2.08	0.13	0.0017
	1st der	2	2.12	0.09	-0.0001
	2nd der	2	2.11	0.09	-0.0001
	1st der + SNV	1	2.11	0.09	-0.0004
	2nd der + SNV	1	2.11	0.10	-0.0008
β-glucan (mg/L)	none	8	102.50	0.12	-0.1552
	mean norm	7	102.37	0.12	-0.2193
	SNV	8	102.50	0.12	0.0414
	1st der	6	102.19	0.13	-0.0744
	2nd der	7	102.76	0.14	0.5394
	1st der + SNV	5	56.86	0.16	-0.5471
	2nd der + SNV	3	104.61	0.08	0.0535

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative; 2nd der=Savitzky-Golay second derivative

Table 17 Summary of the NIR prediction results for whole grain irrigation barley as obtained with full cross-validation for combined 2008 and 2009 data, spectra recorded with the Büchi NIRLab N-200

Property	Pretreatment	Full cross-validation			
		PLS Factors	SECV	R^2	Bias
Plumpness (%)	none	12	4.03	0.54	-0.0020
	mean norm	11	4.08	0.53	0.0062
	SNV	12	3.96	0.56	0.0159
	1st der	9	4.17	0.51	0.0172
	2nd der	8	4.53	0.42	0.0321
	1st der + SNV	9	4.23	0.50	0.0182
	2nd der + SNV	8	4.67	0.40	0.1556
Moisture (%)	none	6	0.56	0.31	0.0004
	mean norm	5	0.56	0.18	0.0020
	SNV	4	0.55	0.19	-0.0003
	1st der	3	0.56	0.18	0.0006
	2nd der	4	0.57	0.16	0.0012
	1st der + SNV	5	0.56	0.18	0.0012
	2nd der + SNV	4	0.57	0.32	0.0011
Extract (%)	none	5	0.95	0.07	-0.0025
	mean norm	4	0.95	0.07	-0.0022
	SNV	3	0.97	0.04	0.0003
	1st der	1	0.96	0.05	0.00001
	2nd der	1	0.96	0.06	0.00003
	1st der + SNV	1	0.96	0.04	-0.0006
	2nd der + SNV	2	0.95	0.06	0.0005
TN (%)	none	11	0.14	0.45	0.0001
	mean norm	11	0.14	0.46	0.0003
	SNV	10	0.14	0.45	0.00002
	1st der	8	0.14	0.45	0.0008
	2nd der	6	0.15	0.45	0.0013
	1st der + SNV	7	0.14	0.43	0.0008
	2nd der + SNV	5	0.15	0.39	0.0002
TSN (%)	none	10	0.08	0.63	0.0004
	mean norm	9	0.08	0.63	0.0004
	SNV	8	0.08	0.62	0.0002
	1st der	7	0.08	0.62	0.0006
	2nd der	5	0.08	0.62	0.0002
	1st der + SNV	6	0.08	0.62	0.0004
	2nd der + SNV	5	0.08	0.62	0.00005
KI	none	8	3.39	0.56	0.0035
	mean norm	7	3.41	0.56	0.0018
	SNV	7	3.51	0.53	0.0043
	1st der	7	3.45	0.55	0.0139
	2nd der	5	3.61	0.51	0.0048
	1st der + SNV	6	3.45	0.55	0.0133
	2nd der + SNV	4	3.59	0.51	0.0189
FAN (mg/L)	none	10	26.90	0.48	0.1717
	mean norm	5	27.92	0.43	0.0339
	SNV	9	26.94	0.47	0.1336
	1st der	7	26.93	0.48	0.1702
	2nd der	5	27.63	0.45	-0.0019
	1st der + SNV	6	27.27	0.46	0.1769
	2nd der + SNV	4	27.66	0.44	0.0427

Table 17 continued

DP (W.K.)	none	6	78.30	0.30	-0.0609
	mean norm	5	78.66	0.29	-0.0908
	SNV	5	80.04	0.27	-0.2019
	1^{st} der	5	76.92	0.33	-0.3719
	2^{nd} der	5	74.05	0.38	-0.2396
	1^{st} der + SNV	6	75.31	0.36	-0.0337
	2^{nd} der + SNV	5	74.39	0.37	-0.2967
Viscosity (cP)	none	9	0.02	0.46	-0.00002
	mean norm	9	0.02	0.47	-0.00003
	SNV	6	0.02	0.48	-0.0001
	1^{st} der	8	0.02	0.46	-0.0002
	2^{nd} der	8	0.02	0.50	-0.0001
	1^{st} der + SNV	6	0.02	0.46	-0.00009
	2^{nd} der + SNV	6	0.02	0.49	-0.00008
AAL	none	14	1.68	0.49	-0.0038
	mean norm	14	1.70	0.48	0.0051
	SNV	12	1.71	0.47	0.0050
	1^{st} der	9	1.69	0.48	0.0047
	2^{nd} der	8	1.75	0.45	0.0013
	1^{st} der + SNV	7	1.71	0.46	0.0010
	2^{nd} der + SNV	8	1.74	0.45	0.0015
β-glucan (mg/L)	none	4	40.48	0.15	0.0287
	mean norm	3	40.34	0.16	-0.0095
	SNV	4	40.20	0.16	0.0085
	1^{st} der	3	40.41	0.16	-0.0501
	2^{nd} der	2	40.42	0.15	0.0111
	1^{st} der + SNV	4	39.77	0.18	-0.0826
	2^{nd} der + SNV	2	40.29	0.16	-0.0157

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate; 1^{st} der=Savitzky-Golay first derivative; 2^{nd} der=Savitzky-Golay second derivative

Table 18 Summary of the NIR prediction results for ground irrigation barley as obtained with full cross-validation for combined 2008 and 2009 data, spectra recorded with the Büchi NIRLab N-200

Property	Pretreatment	Full cross-validation			
		PLS Factors	SECV	R^2	Bias
Plumpness (%)	none	10	4.93	0.32	-0.0035
	mean norm	9	4.87	0.33	-0.0079
	SNV	10	4.94	0.32	-0.0086
	1st der	7	4.84	0.35	0.0034
	2nd der	6	4.84	0.35	0.0100
	1st der + SNV	7	4.83	0.35	-0.0027
	2nd der + SNV	6	4.84	0.35	0.0058
Moisture (%)	none	4	0.50	0.34	-0.0008
	mean norm	3	0.50	0.33	0.0004
	SNV	6	0.50	0.34	-0.0013
	1st der	4	0.51	0.33	-0.0002
	2nd der	7	0.52	0.32	-0.0059
	1st der + SNV	6	0.51	0.32	-0.0026
	2nd der + SNV	3	0.54	0.23	-0.0018
Extract (%)	none	10	0.83	0.30	0.0010
	mean norm	8	0.84	0.27	0.0020
	SNV	7	0.82	0.31	-0.0011
	1st der	5	0.84	0.29	0.0043
	2nd der	5	0.88	0.22	0.0058
	1st der + SNV	5	0.84	0.27	0.0045
	2nd der + SNV	4	0.87	0.23	0.0057
TN (%)	none	5	0.14	0.46	-0.0001
	mean norm	4	0.14	0.46	0.0005
	SNV	3	0.14	0.46	-0.0001
	1st der	4	0.13	0.51	0.0001
	2nd der	3	0.13	0.52	0.0001
	1st der + SNV	3	0.13	0.51	0.0002
	2nd der + SNV	2	0.13	0.53	0.0002
TSN (%)	none	5	0.08	0.60	0.000002
	mean norm	4	0.08	0.62	-0.0008
	SNV	6	0.08	0.58	0.0007
	1st der	5	0.08	0.59	0.0009
	2nd der	2	0.08	0.57	0.0005
	1st der + SNV	5	0.08	0.58	0.0011
	2nd der + SNV	1	0.08	0.56	0.0005
KI	none	3	3.70	0.48	-0.0034
	mean norm	5	3.58	0.51	0.0060
	SNV	5	3.64	0.49	-0.0625
	1st der	6	3.78	0.46	0.0369
	2nd der	4	3.95	0.41	0.0386
	1st der + SNV	4	3.73	0.47	0.0237
	2nd der + SNV	2	3.97	0.40	0.0217
FAN (mg.L)	none	8	27.49	0.45	0.0541
	mean norm	5	27.89	0.43	-0.0105
	SNV	7	27.54	0.45	0.1643
	1st der	5	28.30	0.42	0.3135
	2nd der	2	28.91	0.39	0.1137
	1st der + SNV	5	27.97	0.43	0.3563
	2nd der + SNV	1	28.99	0.39	0.1317

Table 18 continued

DP (W.K.)	none	7	69.54	0.45	0.3841
	mean norm	7	68.46	0.47	0.4255
	SNV	11	69.21	0.46	-0.6341
	1st der	5	72.40	0.40	1.1343
	2nd der	4	71.35	0.42	0.7016
	1st der + SNV	5	71.69	0.42	1.1784
	2nd der + SNV	3	72.27	0.40	0.8759
Viscosity (cP)	none	4	0.02	0.44	0.00001
	mean norm	10	0.02	0.53	0.00020
	SNV	11	0.02	0.50	-0.00010
	1st der	7	0.02	0.51	-0.00021
	2nd der	6	0.02	0.49	-0.00023
	1st der + SNV	6	0.02	0.50	-0.00019
	2nd der + SNV	6	0.02	0.49	-0.00023
AAL	none	8	1.87	0.36	0.0063
	mean norm	8	1.85	0.37	0.0033
	SNV	8	1.95	0.31	0.0156
	1st der	5	1.95	0.30	0.0171
	2nd der	4	1.96	0.29	0.0153
	1st der + SNV	5	1.94	0.31	0.0166
	2nd der + SNV	4	1.95	0.31	0.0184
β-glucan (mg/L)	none	4	40.37	0.16	-0.0706
	mean norm	4	38.64	0.22	-0.8789
	SNV	8	39.12	0.22	-0.4375
	1st der	9	38.07	0.28	-0.4884
	2nd der	3	40.72	0.15	-0.2767
	1st der + SNV	9	38.24	0.28	-0.4758
	2nd der + SNV	6	39.43	0.23	-0.5473

TN=total nitrogen; TSN=total soluble nitrogen, KI=Kolbach index; FAN=free amino nitrogen; DP=diastatic power; W.K.=Windisch Kolbach units; cP=centipoises; AAL=apparent attenuation limit; R^2=coefficient of determination (calibration or cross validation); SECV=standard error of cross validation; RMSECV=root mean square error of cross validation; r^2=coefficient of determination (validation); SEP=standard error of prediction; RMSEP=root mean square error of prediction; RPD=Ratio of (standard error of) Prediction (Validation) to (standard) Deviation; SNV=standard normal variate; 1st der=Savitzky-Golay first derivative; 2nd der=Savitzky-Golay second derivative

Appendix 2

Table 1 Extract (%) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	79.3	78.8	79.3	80.8	79.7	79.4
M2	80.1	79.3	80.9	81.0	80.5	79.3
M3	79.1	79.6	80.6	80.3	80.1	79.7
M4	79.9	79.0	81.3	80.7	79.7	79.5
M5	80.6	80.6	80.7	81.0	80.5	81.0
M6	80.1	79.5	81.0	79.8	79.5	80.4
M7	81.9	81.1	81.7	82.4	81.8	82.1
M8	81.2	80.0	82.1	81.9	81.0	81.7
M9	80.9	81.2	82.7	81.5	80.7	80.2
M10	79.8	80.4	81.1	80.0	79.5	79.6
M11	79.8	79.6	80.6	79.5	80.0	80.0
M12	80.7	80.1	82.1	81.6	80.8	81.4
M13	78.9	80.8	80.8	81.8	80.8	80.5
M14	80.8	79.2	81.5	80.2	79.5	79.6
M15	79.7	78.4	79.7	79.4	79.2	79.5

Table 2 Total nitrogen (%) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	1.46	1.84	1.65	1.61	1.76	1.48
M2	1.68	2.00	1.74	1.60	2.00	1.69
M3	1.58	1.82	1.66	1.66	1.94	1.69
M4	1.55	1.97	1.56	1.56	1.99	1.67
M5	1.41	1.79	1.63	1.55	1.91	1.56
M6	1.42	1.86	1.33	1.59	1.63	1.36
M7	1.33	1.69	1.37	1.37	1.65	1.39
M8	1.53	2.03	1.41	1.56	1.80	1.50
M9	1.55	1.74	1.36	1.51	1.84	1.65
M10	1.50	1.71	1.42	1.70	1.65	1.56
M11	1.49	1.78	1.62	1.71	1.73	1.56
M12	1.55	1.81	1.55	1.64	1.77	1.58
M13	1.66	1.83	1.57	1.63	1.78	1.54
M14	1.55	1.98	1.61	1.82	1.96	1.62
M15	1.61	2.05	1.61	1.71	1.95	1.61

Table 3 Total soluble nitrogen (%) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	0.65	0.78	0.64	0.72	0.78	0.64
M2	0.76	0.84	0.71	0.72	0.92	0.72
M3	0.70	0.83	0.69	0.69	0.88	0.80
M4	0.70	0.89	0.70	0.75	0.92	0.73
M5	0.63	0.78	0.67	0.69	0.85	0.68
M6	0.52	0.64	0.50	0.58	0.63	0.49
M7	0.58	0.77	0.56	0.69	0.75	0.66
M8	0.74	0.82	0.62	0.75	0.77	0.65
M9	0.70	0.81	0.58	0.72	0.88	0.76
M10	0.71	0.79	0.65	0.74	0.82	0.74
M11	0.55	0.64	0.55	0.62	0.68	0.56
M12	0.65	0.95	0.58	0.69	0.66	0.60
M13	0.78	0.76	0.70	0.67	0.69	0.61
M14	0.64	0.89	0.60	0.79	0.86	0.73
M15	0.67	0.82	0.60	0.64	0.77	0.61

Table 4 Kolbach Index values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	44	42	39	45	44	43
M2	45	42	41	45	46	43
M3	44	46	42	42	45	47
M4	45	45	45	48	46	44
M5	44	44	41	44	44	44
M6	37	34	38	36	39	36
M7	44	46	41	50	45	47
M8	48	40	44	48	43	43
M9	45	47	43	48	48	46
M10	47	46	46	43	50	47
M11	37	36	34	36	39	36
M12	42	52	37	42	37	38
M13	47	41	45	41	39	40
M14	41	45	37	43	44	45
M15	42	40	37	37	39	38

Table 5 Free amino nitrogen (mg/L) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	172	218	165	202	209	183
M2	205	234	183	197	262	202
M3	203	252	187	185	263	250
M4	203	273	194	203	284	216
M5	171	221	167	184	232	191
M6	124	154	107	135	163	117
M7	167	229	139	192	216	198
M8	218	236	157	219	232	192
M9	184	259	149	196	283	215
M10	176	231	175	194	245	200
M11	117	170	117	145	185	134
M12	159	244	137	178	187	155
M13	206	213	188	168	189	155
M14	152	286	144	213	254	217
M15	42	40	37	37	39	38

Table 6 Diastatic power (W.K.) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	315	514	335	436	498	420
M2	179	429	342	321	413	364
M3	193	510	385	451	432	471
M4	255	505	370	362	492	444
M5	173	527	385	394	511	416
M6	200	497	350	441	460	378
M7	181	560	400	349	498	405
M8	208	563	416	419	458	396
M9	356	439	352	386	514	443
M10	438	581	387	469	546	468
M11	252	364	286	324	476	373
M12	375	595	407	405	632	628
M13	246	552	293	416	602	635
M14	353	413	423	289	416	388
M15	354	620	402	384	560	481

Table 7 Wort viscosity (cP) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	1.48	1.48	1.52	1.45	1.50	1.49
M2	1.48	1.48	1.49	1.46	1.49	1.48
M3	1.49	1.48	1.48	1.47	1.50	1.46
M4	1.50	1.46	1.44	1.47	1.48	1.45
M5	1.49	1.46	1.44	1.42	1.44	1.44
M6	1.53	1.52	1.49	1.50	1.50	1.51
M7	1.49	1.46	1.46	1.46	1.47	1.46
M8	1.46	1.46	1.42	1.45	1.64	1.45
M9	1.47	1.47	1.46	1.45	1.44	1.45
M10	1.46	1.49	1.46	1.46	1.45	1.45
M11	1.49	1.50	1.53	1.46	1.48	1.50
M12	1.47	1.46	1.48	1.46	1.47	1.47
M13	1.47	1.47	1.46	1.46	1.47	1.46
M14	1.47	1.46	1.50	1.47	1.47	1.48
M15	1.47	1.46	1.51	1.46	1.47	1.48

Table 8 Apparent attenuation limit values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	85.3	80.7	83.4	83.6	83.9	83.8
M2	83.7	83.5	82.8	82.3	82.5	82.2
M3	87.4	83.2	86.2	85.4	85.0	85.8
M4	88.5	82.9	86.6	85.5	85.0	82.3
M5	84.9	81.9	83.5	84.4	83.1	86.0
M6	84.6	86.1	82.7	83.8	83.6	83.8
M7	89.8	80.2	86.8	88.7	86.8	87.2
M8	84.1	82.7	83.3	82.7	81.4	83.0
M9	84.6	83.0	84.9	85.0	85.4	83.4
M10	88.4	85.9	87.5	86.7	87.3	85.5
M11	84.6	83.6	84.3	84.6	83.4	83.3
M12	85.6	83.1	84.7	84.9	83.9	83.6
M13	85.7	84.0	83.4	85.0	83.9	83.9
M14	85.8	82.5	79.9	83.6	83.3	83.1
M15	86	82.4	78.2	83.1	83.6	83.8

Table 9 Wort β-glucan (mg/L) values obtained for dry land 2008 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	70	72	283	70	146	88
M2	52	70	202	59	63	81
M3	49	63	118	65	59	74
M4	69	66	61	65	61	73
M5	82	68	121	65	72	75
M6	90	70	80	91	66	72
M7	81	71	135	80	81	85
M8	57	72	62	69	59	72
M9	71	73	62	67	56	83
M10	69	65	110	71	55	84
M11	110	64	329	99	85	110
M12	61	99	225	62	82	100
M13	60	93	120	55	83	106
M14	75	87	301	73	128	127
M15	57	91	342	87	113	132

Table 10 Extract (%) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	83.7	76.7	77.2	79.3	77.7	76.2
M2	83.1	78.2	80.6	81.4	81.2	79.4
M3	81.9	79.1	79.6	80.9	78.9	78.2
M4	82.0	78.9	80.3	81.0	81.3	78.6
M6	81.6	79.7	79.3	80.1	79.7	78.3
M7	82.6	80.4	80.9	81.6	82.1	80.2
M8	83.8	80.2	81.5	82.0	79.3	79.8
M10	81.7	79.9	79.4	80.0	77.7	79.5
M13	81.7	81.1	81.6	82.6	81.5	80.7

Table 11 Total nitrogen (%) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	1.22	2.57	1.83	1.82	2.17	2.07
M2	1.21	2.49	1.36	1.64	1.84	1.96
M3	1.04	2.18	1.44	1.61	1.94	2.02
M4	1.08	1.67	1.53	1.50	1.22	1.94
M6	1.19	1.85	1.54	1.57	1.71	1.86
M7	1.05	1.80	1.42	1.30	1.87	1.80
M8	1.12	2.05	1.38	1.57	1.52	2.02
M10	1.16	2.26	1.47	1.69	2.16	1.80
M13	1.16	1.71	1.38	1.47	1.87	1.75

Table 12 Total soluble nitrogen (%) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	0.66	1.00	0.80	0.90	0.85	0.76
M2	0.65	1.19	0.66	0.94	0.76	0.75
M3	0.53	1.01	0.69	0.92	0.77	0.93
M4	0.57	0.95	0.79	0.88	0.72	0.96
M6	0.59	0.84	0.64	0.87	0.53	0.57
M7	0.50	0.82	0.66	0.77	0.51	0.67
M8	0.58	1.09	0.66	0.86	0.79	0.81
M10	0.62	1.05	0.68	0.93	0.83	0.79
M13	0.65	0.89	0.62	0.77	0.68	0.74

Table 13 Kolbach Index values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	54	39	44	49	39	37
M2	54	48	48	57	41	38
M3	51	49	48	57	40	46
M4	53	57	52	59	59	49
M6	50	45	42	55	31	31
M7	48	45	46	59	27	37
M8	52	43	48	55	52	40
M10	83	46	46	55	38	44
M13	56	58	45	52	36	42

Table 14 Free amino nitrogen (mg/L) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	164	210	213	230	192	181
M2	179	277	187	254	171	179
M3	149	251	209	257	144	259
M4	158	252	243	261	182	252
M6	140	171	154	210	111	131
M7	124	180	166	200	124	144
M8	154	267	75	244	180	179
M10	152	223	185	248	185	163
M13	216	216	189	208	149	150

Table 15 Diastatic power (W.K.) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	261	738	494	478	267	324
M2	197	520	325	317	277	317
M3	258	584	400	356	283	319
M4	235	354	420	338	279	323
M6	208	458	318	392	279	318
M7	248	556	374	458	275	320
M8	245	501	335	405	299	327
M10	298	661	367	506	297	325
M13	317	404	383	453	292	327

Table 16 Wort viscosity (cP) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	1.64	1.45	1.52	1.45	1.51	1.50
M2	1.50	1.46	1.50	1.45	1.51	1.48
M3	1.48	1.47	1.53	1.48	1.52	1.46
M4	1.48	1.47	1.50	1.46	1.47	1.44
M6	1.65	1.49	1.59	1.50	1.55	1.55
M7	1.51	1.46	1.53	1.47	1.66	1.59
M8	1.52	1.47	1.49	1.47	1.46	1.45
M10	1.49	1.48	1.50	1.47	1.48	1.50
M13	1.54	1.48	1.64	1.47	1.50	1.47

Table 17 Apparent attenuation limit values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	83.1	83.1	84.1	87.30	85.4	79.5
M2	82.7	84.1	85.8	84.80	87.7	79.6
M3	88.4	85.7	88.4	84.0	81.7	83.7
M4	87.1	85.7	88.2	86.6	83.2	85.0
M6	87.7	85.0	83.2	84.3	82.0	78.7
M7	81.8	87.3	88.2	88.9	85.1	81.5
M8	87.8	82.3	84.7	86.0	83.9	81.5
M10	81.6	85.1	87.0	88.2	84.3	85.7
M13	87.0	85.2	85.8	86.1	84.8	85.0

Table 18 Wort β-glucan (mg/L) values obtained for dry land 2009 localities

Line	Napier	Klipdale	Bredasdorp	Caledon	Swellendam	Heidelberg
M1	53	47	63	39	283	306
M2	43	33	37	32	96	208
M3	37	28	24	28	144	143
M4	73	28	22	28	32	137
M6	29	32	53	26	305	442
M7	24	40	46	52	822	597
M8	105	43	28	33	142	152
M10	174	36	47	24	130	153
M13	150	40	19	40	205	137

Table 19 Extract (%) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	80.1	81.2	81.8	81.0	81.3
M17	80.9	81.6	81.7	81.1	82.3
M18	80.4	83.6	82.6	81.7	82.3
M19	80.4	82.1	80.9	80.8	81.6
M20	80.2	83.4	81.5	82.0	81.6
M21	80.1	83.2	81.2	82.0	82.2
M22	80.1	81.3	81.7	82.3	81.2
M23	80.7	82.1	80.4	82.3	81.4
M24	79.5	79.7	81.3	82.0	81.0
M25	81.2	82.2	80.9	82.7	81.9
M3	79.5	80.6	81	81.8	80.3
M26	79.3	80.6	80.1	81.8	81.6

Table 20 Total nitrogen (%) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	1.64	1.64	1.69	1.46	1.68
M17	1.56	1.53	1.82	1.56	1.49
M18	1.50	1.36	1.70	1.39	1.40
M19	1.61	1.50	1.65	1.58	1.54
M20	1.50	1.34	1.79	1.47	1.59
M21	1.53	1.33	1.66	1.37	1.36
M22	1.71	1.67	1.86	1.56	1.48
M23	1.50	1.56	1.73	1.56	1.52
M24	1.83	1.75	1.99	1.52	1.90
M25	1.35	1.28	1.66	1.28	1.51
M3	1.67	1.53	1.87	1.45	1.69
M26	1.58	1.46	1.66	1.44	1.41

Table 21 Total soluble nitrogen (%) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	0.67	0.68	0.69	0.53	0.84
M17	0.73	0.64	0.62	0.58	0.76
M18	0.59	0.59	0.64	0.52	0.64
M19	0.61	0.60	0.62	0.58	0.65
M20	0.56	0.55	0.63	0.60	0.61
M21	0.59	0.56	0.81	0.50	0.58
M22	0.78	0.79	0.66	0.63	0.71
M23	0.69	0.66	0.96	0.64	0.69
M24	0.80	0.73	0.54	0.68	0.83
M25	0.55	0.52	0.86	0.46	0.56
M3	0.79	0.76	0.53	0.55	0.80
M26	0.60	0.50	0.66	0.58	0.54

Table 22 Kolbach Index values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	41	41	37	36	50
M17	47	42	38	37	51
M18	39	43	36	38	40
M19	38	40	39	37	42
M20	37	41	35	41	38
M21	39	42	38	36	43
M22	46	47	43	40	48
M23	46	42	38	41	45
M24	44	48	38	45	44
M25	41	41	32	36	37
M3	47	50	46	38	47
M26	38	34	32	40	38

Table 23 Free amino nitrogen (mg/L) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	186	156	153	123	245
M17	217	143	133	143	224
M18	149	126	132	125	167
M19	152	124	119	139	164
M20	140	119	137	149	142
M21	153	134	184	121	144
M22	224	183	142	160	187
M23	196	167	155	166	178
M24	215	166	111	174	217
M25	143	129	195	115	140
M3	216	210	108	134	235
M26	138	99	150	111	132

Table 24 Diastatic power (W.K.) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	554	399	254	330	435
M17	402	225	278	340	242
M18	408	212	398	277	265
M19	459	357	361	378	400
M20	382	279	366	376	417
M21	387	278	353	410	376
M22	420	322	362	439	379
M23	435	284	356	414	408
M24	462	332	358	334	389
M25	403	281	319	359	420
M3	433	236	220	306	235
M26	324	198	228	510	260

Table 25 Wort viscosity (cP) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	1.45	1.45	1.52	1.49	1.47
M17	1.48	1.47	1.48	1.49	1.50
M18	1.45	1.44	1.47	1.47	1.45
M19	1.44	1.44	1.49	1.48	1.46
M20	1.48	1.46	1.47	1.47	1.48
M21	1.47	1.45	1.54	1.46	1.47
M22	1.46	1.44	1.50	1.47	1.48
M23	1.45	1.45	1.52	1.46	1.47
M24	1.47	1.46	1.47	1.49	1.46
M25	1.45	1.46	1.60	1.46	1.43
M3	1.48	1.48	1.53	1.48	1.54
M26	1.50	1.53	1.49	1.45	1.48

Table 26 Apparent attenuation limit values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	84.7	80.4	79.4	77.6	83.1
M17	81.5	78.0	78.3	81.8	78.7
M18	84.4	80.7	80.9	81.4	82.2
M19	84.7	84.0	83.0	82.9	84.1
M20	85.0	83.1	80.9	81.2	84.0
M21	85.0	85.1	83.6	80.2	84.8
M22	85.5	84.7	82.6	82.2	82.7
M23	84.5	82.0	80.7	81.2	81.6
M24	84.4	84.3	80.9	77.0	81.9
M25	86.3	84.3	84.4	85.5	85.0
M3	88.0	83.7	83.8	80.6	80.7
M26	82.2	83.3	79.1	85.4	77.3

Table 27 Wort β-glucan (mg/L) values obtained for irrigation 2008 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	86	97	293	158	37
M17	69	132	176	75	37
M18	68	76	212	82	39
M19	76	125	223	78	35
M20	75	81	79	177	37
M21	71	66	188	64	39
M22	79	78	405	159	58
M23	72	129	423	102	79
M24	130	199	191	180	91
M25	70	112	195	113	49
M3	76	119	439	136	41
M26	75	326	87	111	51

Table 28 Extract (%) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	81.9	81.5	81.6	80.0	80.1
M17	80.8	81.3	81.9	81.5	81.2
M18	81.9	82.9	83.5	80.3	80.9
M19	81.4	81.9	81.5	80.7	81.6
M21	82.0	80.5	81.6	81.3	81.8
M22	81.7	81.3	82.7	81.8	80.8
M23	82.2	72.4	81.3	79.9	81.4
M25	81.4	82.0	82.2	80.8	79.8

Table 29 Total nitrogen (%) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	1.52	1.72	1.74	1.82	2.07
M17	1.67	1.84	1.80	1.83	2.03
M18	1.50	1.53	1.67	1.83	1.90
M19	1.58	1.62	1.86	1.77	1.89
M21	1.39	1.78	1.83	1.59	1.73
M22	1.51	1.90	1.50	1.56	2.01
M23	1.42	1.71	1.92	1.66	1.93
M25	1.39	1.76	1.59	1.76	1.94

Table 30 Total soluble nitrogen (%) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	0.84	0.84	0.91	0.86	0.98
M17	0.88	0.97	0.84	0.79	0.94
M18	0.73	0.70	0.90	0.77	0.83
M19	0.73	0.86	0.84	0.77	0.86
M21	0.68	0.91	0.85	0.86	0.86
M22	0.72	0.87	0.75	0.88	0.91
M23	0.83	0.92	0.94	0.78	0.93
M25	0.66	0.80	0.74	0.84	0.85

Table 31 Kolbach Index values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	55	49	52	46	47
M17	52	53	46	43	46
M18	48	46	43	42	44
M19	46	53	45	43	46
M21	49	51	46	54	49
M22	48	46	50	56	45
M23	55	54	49	47	48
M25	47	45	46	48	44

Table 32 Free amino nitrogen (mg/L) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	212	209	241	174	249
M17	231	260	240	214	238
M18	192	180	235	199	221
M19	180	224	223	176	218
M21	178	235	207	187	210
M22	176	213	173	220	204
M23	197	251	208	194	202
M25	166	189	163	167	191

Table 33 Diastatic power (W.K.) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	445	479	476	436	595
M17	441	452	400	563	393
M18	375	459	394	537	394
M19	593	534	673	472	479
M21	388	480	462	483	407
M22	384	555	409	513	464
M23	363	567	534	486	496
M25	366	602	477	446	543

Table 34 Wort viscosity (cP) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	1.39	1.40	1.40	1.44	1.44
M17	1.42	1.49	1.40	1.45	1.46
M18	1.40	1.41	1.42	1.47	1.45
M19	1.40	1.40	1.39	1.46	1.45
M21	1.4	1.41	1.41	1.44	1.46
M22	1.40	1.43	1.48	1.45	1.47
M23	1.41	1.42	1.44	1.43	1.48
M25	1.39	1.41	1.47	1.45	1.44

Table 35 Apparent attenuation limit values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	86.7	84.9	85.4	83.0	82.7
M17	83.2	86.5	83.6	86.2	80.5
M18	86.0	87.9	86.4	84.9	83.0
M19	87.6	87.7	87.2	84.7	84.1
M21	88.6	86.2	84.1	86.8	84.6
M22	85.6	85.9	86.8	87.1	81.0
M23	87.5	87.6	84.8	85.8	82.9
M25	84.9	86.4	86.5	83.6	81.8

Table 36 Wort β-glucan (mg/L) values obtained for irrigation 2009 localities

Line	Luckhoff	Douglas	Rietrivier	Hartswater	Taung
M16	46	57	41	144	58
M17	54	73	42	98	76
M18	37	56	32	90	52
M19	48	53	28	169	59
M21	29	60	30	125	44
M22	61	86	49	71	104
M23	68	60	60	66	134
M25	48	59	84	155	48